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A

Date: April 23, 1999

Re: 454312-3140

ASSISTANT COMMISSIONER FOR PATENTS
Box Patent Application (35 U.S.C. 111)
Washington, D.C. 20231

Sir:

With reference to the filing in the United States Patent and Trademark Office of a Continuation application of a PCT application, pursuant to 35 U.S.C. 111(a) in the name of:

David E. BRILES, Susan K. HOLLINGSHEAD and Alexis BROOKS-WALTER

entitled:

**PNEUMOCOCCAL SURFACE PROTEIN C (PspC), EPITOPIC REGIONS
AND STRAIN SELECTION THEREOF, AND USES THEREFOR**

The following are enclosed:

- ☒ Specification (44 pages) Abstract (page 48)
☒ 50 Sheet(s) of Drawings, (Figures 1-21)
☒ 27 Claim(s) (including 1 independent claim(s); via multiple dependencies, 72 claims, with 1 independent claim; pages 45-47)

- ☒ This application contains multiply dependent claims
☒ Oath or Declaration and Power of Attorney ☒ signed ☐ unsigned
☐ Preliminary Amendment

- ☒ Our checks for \$2,100.00, calculated as follows:

Basic Fee, \$760.00 (\$380.00).....	\$ 760.00
Number of Claims in excess of 20 at \$18.00 (\$9.00) each:	1,070.00
Number of Independent Claims in excess of 3 at \$78.00 (\$39.00) each:	-0-
Multiple Dependent Claim Fee at \$260.00 (\$130.00)	260.00
Total Filing Fee	\$2,060.00
Assignment Recording Fee \$40.00	40.00

- ☐ Pursuant to MPEP § 1895, enclosed is a copy of the PCT Request which is submitted as evidence that the instant continuation is copending with the PCT application and has at least one inventor in common therewith.

- ☐ Priority is claimed from each of the following application(s):

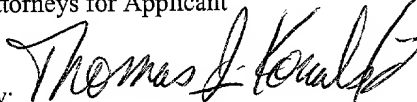
<u>Application No.</u>	<u>Filed</u>	<u>In</u>
60/082,728	4/23/98	U.S.

The undersigned attorney of record, Thomas J.Kowalski, Reg. No. 32,147, hereby appoints William S. Frommer, Reg. No. 25,506, his associate attorney, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the patent, and to transact all business in the U.S. Patent and Trademark Office and in the courts in connection therewith, and specify that all written communications about the application are to be directed to the following address:

William S. Frommer
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New York, NY 10151

Please charge any additional fees required for the filing of this application or credit any overpayment to Deposit Account No. 50-0320.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP
Attorneys for Applicant

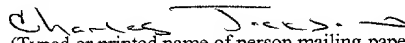
By: 
Thomas J. Kowalski
Registration No. 32,147

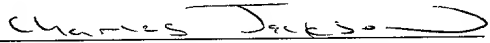
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PATENT
454312-3140

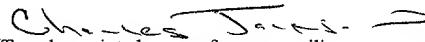
IN THE UNITED STATES PATENT AND TRADEMARK
APPLICATION FOR LETTERS PATENT

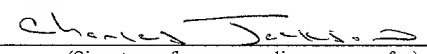
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TITLE: PNEUMOCOCCAL SURFACE PROTEIN C (PspC), EPITOPIC
REGIONS AND STRAIN SELECTION THEREOF, AND USES
THEREFOR

INVENTORS: David E. BRILES, Susan K. HOLLINGSHEAD, and Alexis BROOKS-
WALTER

SPECIFICATION: 44 pages (with 1 page abstract)

CLAIMS: 27 (1 independent; via multiple dependencies, 72 claims)

DRAWINGS: 50 sheets (Figures 1 to 21)

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TITLE OF THE INVENTION

***PNEUMOCOCCAL SURFACE PROTEIN C (PspC), EPITOPIC REGIONS
AND STRAIN SELECTION THEREOF, AND USES THEREFOR***

STATEMENT OF GOVERNMENT SUPPORT

This work was supported in part by National Institute of Health Grants AI21548 and HL58418.

RELATED APPLICATIONS/PATENTS

This application is based upon and claims priority from U.S. Provisional application Serial No. 60/082,728, filed April 23, 1998.

Reference also is made to: Briles et al., "Strain Selection of Pneumococcal Surface Proteins," U.S. application Serial No. 08/710,749, filed September 20, 1996 (allowed); Briles et al., "Pneumococcal Genes, Portions Thereof, Expression Products Therefrom, And Uses of Such Genes, Portions and Products," U.S. applications Serial Nos. 08/714,741, filed September 16, 1996, and 08/529,055, filed September 15, 1995, and PCT applications PCT/US96/14819, filed September 16, 1996 and WO 97/09994, published March 20, 1997; Briles et al. "Oral Administration ...," U.S. application Serial Nos. 08/482,981, filed June 7, 1995 (allowed); U.S. application Serial No. 08/458,399, filed June 2, 1995, and U.S. application Serial No. 08/657,751, filed May 30, 1996 (allowed); "Mucosal Administration ...," Briles et al., U.S. application Serial No. 08/446,201, filed May 19, 1995 (allowed; filed as a CIP of USSN 08/246,636, filed May 20, 1994 (also allowed)), and Briles et al., U.S. application Serial No. 08/312,949, filed September 30, 1994 (allowed); Briles et al., U.S. application Serial No. 08/319,795, filed May, 20, 1994 (allowed); Briles et al., "Epitopic Regions of Pneumococcal Surface Protein A," U.S. application Serial No. 08/456,746, filed June 6, 1995 (now U.S. Patent No. 5,679,768; filed as a cont. USSN 08/048,896, filed April 20, 1993, now abandoned, which was as a CIP of USSN 07/835,698, filed February 12, 1992, now abandoned, which was as a CIP of USSN 07/656,773, now abandoned); Briles et al., "Structural Gene of Pneumococcal Protein," U.S. application Serial No. 08/467,852, filed June 6, 1995 (now U.S. Patent No. 5,856,170; filed as a cont. of U.S. application Serial No. 08/247,491, filed May 23, 1994), U.S. application Serial No. 08/072,070, filed June 3, 1993 (now U.S. Patent No. 5,476,929) and U.S. Patent Nos. 5,753,463 and 5,728,387 (from U.S. applications Serial Nos. 08/469,434, filed June 6, 1995 and U.S. application Serial No. 214,164, filed March 14, 1994, respectively); Briles et

al., "Truncated PspA ...," U.S. application Serial No. 08/214,222, filed March 17, 1994 (now U.S. Patent No. 5,804,193); Briles et al. U.S. application Serial No. 08/468,985 (allowed); Briles et al., "Immunoassay Comprising a Truncated Pneumococcal Surface Protein A (PspA)," U.S. Patent No. 5,871,943; U.S. applications Serial Nos. 08/226,844, filed May 29, 1992, 08/093,907, filed July 5, 1994, and 07/889,918, filed July 5, 1994; PCT/US93/05191; and Briles et al., WO 92/1448.

Each of these applications and patents, as well as each document or reference cited in each of these applications and patents (including during the prosecution of each issued patent) and PCT and foreign applications or patents corresponding to and/or claiming priority from any of the foregoing applications and patents, is hereby expressly incorporated herein by reference. Documents or references are also cited in the following text, either in a Reference List before the claims, or in the text itself; and, each of these documents or references ("herein-cited documents or references"), as well as each document or reference cited in each of the herein-cited documents or references, is hereby expressly incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to epitopic regions of Pneumococcal Surface Protein C or "PspC", different clades of PspC, isolated and/or purified nucleic acid molecules such as DNA encoding a fragment or portion of PspC such as an epitopic region of PspC or at least one epitope of PspC, uses for such nucleic acid molecules, e.g., to detect the presence of PspC or of *S. pneumoniae* by detecting a nucleic acid molecule therefor in a sample such as by amplification and/or a polymerase chain reaction, vectors or plasmids which contain and/or express such nucleic acid molecules, e.g., *in vitro* or *in vivo*, immunological, immunogenic or vaccine compositions comprising at least one PspC and/or a portion thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide comprising at least one epitope of PspA.

PspC or a fragment thereof, and thus a composition comprising PspC or a fragment thereof, can be administered by the same routes, and in approximately the same amounts, as

PspA. Thus, the invention further provides methods for administering PspC or a fragment thereof, as well as uses of PspC or a fragment thereof to formulate such compositions.

Other aspects of the invention are described in or are obvious from (and within the ambit of the invention) the following disclosure.

BACKGROUND OF THE INVENTION

Streptococcus pneumoniae is an important cause of otitis media, meningitis, bacteremia and pneumonia, and a leading cause of fatal infections in the elderly and persons with underlying medical conditions, such as pulmonary disease, liver disease, alcoholism, sickle cell, cerebrospinal fluid leaks, acquired immune deficiency syndrome (AIDS), and patients undergoing immunosuppressive therapy. It is also a leading cause of morbidity in young children. Pneumococcal infections cause approximately 40,000 deaths in the U.S. yearly. The most severe pneumococcal infections involve invasive meningitis and bacteremia infections, of which there are 3,000 and 50,000 cases annually, respectively.

Despite the use of antibiotics and vaccines, the prevalence of pneumococcal infections has declined little over the last twenty-five years; the case-fatality rate for bacteremia is reported to be 15-20% in the general population, 30-40% in the elderly, and 36% in inner-city African Americans. Less severe forms of pneumococcal disease are pneumonia, of which there are 500,000 cases annually in the U.S., and otitis media in children, of which there are an estimated 7,000,000 cases annually in the U.S. caused by pneumococcus. Strains of drug-resistant *S. pneumoniae* are becoming ever more common in the U.S. and worldwide. In some areas, as many as 30% of pneumococcal isolates are resistant to penicillin. The increase in antimicrobial resistant pneumococcus further emphasizes the need for preventing pneumococcal infections.

Pneumococcus asymptomatically colonizes the upper respiratory tract of normal individuals; disease often results from the spread of organisms from the nasopharynx to other tissues during opportunistic events. The incidence of carriage in humans varies with age and circumstances. Carrier rates in children are typically higher than those of adults. Studies have demonstrated that 38 to 60% of preschool children, 29 to 35% of grammar school children and 9 to 25% of junior high school children are carriers of pneumococcus. Among adults, the rate of carriage drops to 6% for those without children at home, and to 18 to 29% for those with children at home. It is not surprising that the higher rate of carriage in children than in adults parallels the incidence of pneumococcal disease in these populations.

An attractive goal for streptococcal vaccination is to reduce carriage in the vaccinated populations and subsequently reduce the incidence of pneumococcal disease. There is speculation that a reduction in pneumococcal carriage rates by vaccination could reduce the incidence of the disease in non-vaccinated individuals as well as vaccinated individuals. This "herd immunity" induced by vaccination against upper respiratory bacterial pathogens has been observed using the *Haemophilus influenzae* type b conjugate vaccines (Takala, A.K., et al., J. Infect. Dis. 1991; 164: 982-986; Takala, A.K., et al., Pediatr. Infect. Dis. J., 1993; 12: 593-599; Ward, J., et al., Vaccines, S.A. Plotkin and E. A. Mortimer, eds., 1994, pp. 337-386; Murphy, T.V., et al., J. Pediatr., 1993; 122: 517-523; and Mohle-Boetani, J.C., et al., Pediatr. Infect. Dis. J., 1993; 12: 589-593).

It is generally accepted that immunity to *Streptococcus pneumoniae* can be mediated by specific antibodies against the polysaccharide capsule of the pneumococcus. However, neonates and young children fail to make adequate immune response against most capsular polysaccharide antigens and can have repeated infections involving the same capsular serotype. One approach to immunizing infants against a number of encapsulated bacteria is to conjugate the capsular polysaccharide antigens to protein to make them immunogenic. This approach has been successful, for example, with *Haemophilus influenzae b* (see U.S. Patent No. 4,496,538 to Gordon and U.S. Patent No. 4,673,574 to Anderson).

However, there are over ninety known capsular serotypes of *S. pneumoniae*, of which twenty-three account for about 95% of the disease. For a pneumococcal polysaccharide-protein conjugate to be successful, the capsular types responsible for most pneumococcal infections would have to be made adequately immunogenic. This approach may be difficult, because the twenty-three polysaccharides included in the presently-available vaccine are not all adequately immunogenic, even in adults.

Protection mediated by anti-capsular polysaccharide antibody responses are restricted to the polysaccharide type. Different polysaccharide types differentially facilitate virulence in humans and other species. Pneumococcal vaccines have been developed by combining 23 different capsular polysaccharides that are the prevalent types of human pneumococcal disease. These 23 polysaccharide types have been used in a licensed pneumococcal vaccine since 1983 (D.S. Fedson and D. M. Musher, Vaccines, S.A. Plotkin and J.E.A. Montimer, eds., 1994, pp.

517-564). The licensed 23-valent polysaccharide vaccine has a reported efficacy of approximately 60% in preventing bacteremia caused vaccine type pneumococci in healthy adults.

However, the efficacy of the vaccine has been controversial, and at times, the justification for the recommended use of the vaccine questioned. It has been speculated that the efficacy of this vaccine is negatively affected by having to combine 23 different antigens. Having a large number of antigens combined in a single formulation may negatively affect the antibody responses to individual types within this mixture because of antigenic competition. The efficacy is also affected by the fact that the 23 serotypes encompass all serological types associated with human infections and carriage.

An alternative approach to protecting against pneumococcal infection, especially for protecting children, and also the elderly, from pneumococcal infection, would be to identify protein antigens that could elicit protective immune responses. Such proteins may serve as a vaccine by themselves, may be used in conjunction with successful polysaccharide-protein conjugates, or as carriers for polysaccharides.

Pneumococcal Surface Protein A or PspA, has been identified as an antigen; and, its DNA and amino acid sequences have been investigated. PspA is useful in eliciting protective immune responses. PspA or fragments thereof can be used in immunological, immunogenic or vaccine compositions; and, such compositions can contain different types of PspAs or fragments from different types of PspAs. Further, such compositions can be administered by injection, or mucosally or orally, or by means of a vector expressing the PspA or fragment thereof.

Studies on PspA led to the discovery of a PspA-like protein and a *pspA*-like gene, now termed PspC and *pspC*. Indeed, early patent literature termed PspC as "PspA-like".

It is believed that heretofore that epitopic regions of PspC have not been disclosed or suggested. It is likewise believed that heretofore different clades of PspC have not been taught or suggested. Further, it is believed that heretofore DNA encoding epitopic regions of PspC have not been disclosed or suggested. Further still, it is believed that heretofore immunological, immunogenic or vaccine compositions comprising at least one PspC and/or portions thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof

and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide comprising at least one epitope of PspA, have not been taught or suggested.

Alternative vaccination strategies are desirable as such provide alternative immunological, immunogenic or vaccine compositions, as well as alternative routes to administration or alternative routes to responses. It would be advantageous to provide an immunological composition or vaccination regimen which elicits protection against various diversified pneumococcal strains, without having to combine a large number of possibly competitive antigens within the same formulation. And, it is advantageous to provide additional antigens and epitopes for use in immunological, immunogenic and/or vaccine compositions, e.g., to provide alternative compositions containing or comprising such antigens or epitopes either alone or in combination with different antigens.

Furthermore it is advantageous to provide a better understanding of the pathogenic mechanisms of pneumococci, as this can lead to the development of improved vaccines, diagnosis and treatments.

OBJECTS AND SUMMARY OF THE INVENTION

An object of the invention can include providing one or more of: epitopic regions of PspC, different clades of PspC, isolated and/or purified nucleic acid molecules such as DNA encoding a fragment or portion of PspC such as an epitopic region of PspC or at least one epitope of PspC, uses for such nucleic acid molecules, vectors or plasmids which contain and/or express such nucleic acid molecules, e.g., *in vitro* or *in vivo*, immunological, immunogenic or vaccine compositions comprising such a vector or plasmid and/or at least one PspC and/or a portion thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide comprising at least one epitope of PspA and/or at least one vector or plasmid expressing said second pneumococcal antigen (which vector or plasmid could be the same as the aforementioned vector or plasmid comprising a nucleic molecule encoding PspC or a portion or fragment thereof); and, methods for administering PspC or a fragment thereof, as well as uses of PspC or a fragment thereof to formulate such compositions, *inter alia*.

Accordingly, the invention can provide one or more of: epitopic regions of PspC, different clades of PspC, isolated and/or purified nucleic acid molecules such as DNA encoding a fragment or portion of PspC such as an epitopic region of PspC or at least one epitope of PspC, uses for such nucleic acid molecules, vectors or plasmids which contain and/or express such nucleic acid molecules, e.g., *in vitro* or *in vivo*, immunological, immunogenic or vaccine compositions comprising such a vector or plasmid and/or at least one PspC and/or a portion thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide comprising at least one epitope of PspA and/or at least one vector or plasmid expressing said second pneumococcal antigen (which vector or plasmid could be the same as the aforementioned vector or plasmid comprising a nucleic molecule encoding PspC or a portion or fragment thereof); and, methods for administering PspC or a fragment thereof, as well as uses of PspC or a fragment thereof to formulate such compositions, *inter alia*.

PspC or a fragment thereof, and thus a composition comprising PspC or a fragment thereof, can be administered by the same routes, and in approximately the same amounts, as PspA. Thus, the invention further provides methods for administering PspC or a fragment thereof, as well as uses of PspC or a fragment thereof to formulate such compositions.

Still further, the invention provides PspC epitopic regions, e.g., the alpha helical region, or the proline region or the combination of the alpha helical and proline regions, or the entire PspC molecule, or aa 1-590 of PspC clade A, or amino acid(s) ("aa") 1-204 or aa 46-204 or aa 1-295 or aa 46-295 or aa 1-454 or aa 46-454 or aa 204-454 or aa 295-454 or aa 1-590 or aa 46-590 or aa 204-590 or aa 295-590 or aa 454-590 or aa 1-652 or aa 46-652 or aa 204-652 or aa 295-652 or aa 454-652 or aa 590-652 or aa 1-892 or aa 46-892 or aa 204-892 or aa 295-892 or aa 454-892 or 590-892 of PspC clade A. A prototypic clade A PspC is PspC.EF6796. In other clade A PspCs, the epitopic regions may have slightly different amino acid numbers. Thus, the invention comprehends regions of other clade A PspCs which are substantially homologous, or significantly homologous, or highly homologous, or very highly homologous, or identical, or highly conserved, with respect to the foregoing particularly recited epitopic regions. Also, where possible, these regions can extend in either the N-terminal or COOH-terminal direction; e.g., by

about another 1-25 or 1-50 amino acids in either or both directions. The invention further provides a polypeptide comprising at least one epitopic region or at least one epitope in any one of these various regions.

Similarly, the invention provides clade B epitopic regions, e.g., the alpha helical region, the proline region, the combination of the alpha helical and proline regions, and the entire molecule, as well as by aa such as aa 1-664, or aa 1-375, or aa 1-445 or aa 1-101, or aa 1-193, or aa 1-262, or aa 1-355, or aa 101-193, or aa 101-262, or aa 101-355, or aa 101-375, or aa 101-455 or aa 193-262, or aa 193-355, or aa 193-375, or aa 193-445 or aa 262-355, or aa 262-375, or aa 262-445 or aa 355-375, or aa 355-445 or aa 375-445 or aa 101-664, or aa 193-664, or aa 262-664, or aa 355-664 or aa 375-664 or aa 1-end of proline subregion A, or aa 1-beginning of proline subregion B, or aa 101-end of proline subregion A, or aa 101-beginning of proline subregion B, or aa 193-end of proline subregion A, or aa 193-beginning of proline subregion B, or aa 262-end of proline subregion A, or aa 262-beginning of proline subregion B, or aa 355-end of proline subregion A, or aa 355-beginning of proline subregion B, or aa 375-end of proline subregion A, or proline subregion A, or aa 375-beginning of proline subregion B, or proline subregion B, or beginning of proline subregion B-aa 664. A prototypic clade B PspC is PspC.D39. In other clade B PspCs, the epitopic regions may have slightly different amino acid numbers. Thus, the invention comprehends regions of other clade B PspCs which are substantially homologous, or significantly homologous, or highly homologous, or very highly homologous, or identical, or highly conserved, with respect to the foregoing particularly recited epitopic regions. Also, where possible, these regions can extend in either the N-terminal or COOH-terminal direction; e.g., by about another 1-25 or 1-50 amino acids in either or both directions. For instance, interesting epitopic regions include: aa 263-482, 1-445 and 255-445. And, the invention further provides a polypeptide comprising at least one epitopic region or at least one epitope in any one of these regions.

A polypeptide comprising at least one epitope of PspC or PspA can be shorter than natural or full length PspC or PspA, e.g., a truncated PspC or PspA, such as comprising up to about 90% of natural or full length PspC or PspA.

The invention further provides an isolated nucleic acid molecule, e.g., DNA comprising a sequence encoding any one of these epitopic regions or a polypeptide comprising at least one of these epitopic regions, or an epitope of PspC; such a nucleic acid molecule is advantageously at

least about 12 nucleotides in length, for instance, at least about 15, about 18, about 21, about 24 or about 27 nucleotides in length, such as at least about 30, about 33, about 36, about 39 or about 42 nucleotides in length, for example, a nucleic acid molecule of at least about 12 nucleotides in length such as about 12 to about 30, about 12 to about 50 or about 12 to about 60, or about 12 to about 75 or about 12 to about 100 or more nucleotides in length. A nucleic acid molecule comprising a sequence encoding at least one epitope of PspC or PspA can be shorter than natural or full length *pspC* or *pspA*, e.g., a truncated *pspC* or *pspA*, such as comprising up to about 90% of natural or full length *pspC* or *pspA* or encoding up to about 90% of natural or full length PspA or PspC.

Moreover, in this disclosure, Applicants demonstrate cross-reactivity between PspC and PspA, as well as regions of PspC and PspA and/or of *pspC* and *pspA* which are highly conserved, substantially homologous, highly homologous, and identical. This information allows the skilled artisan to identify nucleic acid molecules which can hybridize, e.g., specifically ("specific hybridization") to *pspC* or *pspA* or both *pspC* and *pspA*, e.g., under stringent conditions. The term "specific hybridization" will be understood to mean that the nucleic acid probes of the invention are capable of stable, double-stranded hybridization to bacterially-derived DNA or RNA under conditions of high stringency, as the term "high stringency" would be understood by those with skill in the art (see, for example, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Hames and Higgins, eds., 1985, Nucleic Acid Hybridization, IRL Press, Oxford, U.K.). Hybridization will be understood to be accomplished using well-established techniques, including but not limited to Southern blot hybridization, Northern blot hybridization, *in situ* hybridization and, most preferably, Southern hybridization to PCR-amplified DNA fragments. In a preferred alternative, the nucleic acid hybridization probe of the invention may be obtained by use of the polymerase chain reaction (PCR) procedure, using appropriate pairs of PCR oligonucleotide primers as provided herein or from the teachings herein. See U.S. Pat. Nos. 4,683,195 to Mullis et al. and 4,683,202 to Mullis. A probe or primer can be any stretch of at least 8, preferably at least 10, more preferably at least 12, 13, 14, or 15, such as at least 20, e.g., at least 23 or 25, for instance at least 27 or 30 nucleotides in *pspC* which are unique to *pspC*, e.g., not also in *pspA* (when amplification of just *pspC* is desired) or unique to both *pspC* and *pspA* or in both *pspC* and *pspA* (when amplification of both is acceptable or desired) or which are in *pspC* and are least

conserved among the *pspC/pspA* genes. As to PCR or hybridization primers or probes and optimal lengths therefor, reference is also made to Kajimura et al., GATA 7(4):71-79 (1990). The invention will thus be understood to provide oligonucleotides, such as , pairs of oligonucleotides, for use as primers for the *in vitro* amplification of bacterial DNA samples and fragments thereof, or for use in expressing a portion of bacterial DNA, either *in vitro* or *in vivo*. The oligonucleotides preferably specifically hybridize to sequences flanking a nucleic acid to be amplified, wherein the oligonucleotides hybridize to different and opposite strands of the double-stranded DNA target. The oligonucleotides of the invention are preferably derived from the nucleic acid molecules and teachings herein. As used in the practice of this invention, the term "derived from" is intended to encompass the development of such oligonucleotides from the nucleic acid molecules and teachings disclosed herein, from which a multiplicity of alternative and variant oligonucleotides can be prepared.

And, the invention further comprehends vectors or plasmids containing and/or expressing such a nucleic acid molecule, as well as uses of such nucleic acid molecules, e.g., for expression of PspC or an epitopic region thereof or at least an epitope thereof or a polypeptide comprising at least one epitope thereof either *in vitro* or *in vivo*, or for amplifying or detecting PspC or *S. pneumoniae* in a sample, for instance by a polymerase chain reaction.

With respect to the herein mentioned nucleic acid molecules and polypeptides, e.g., the aforementioned nucleic acid molecules and polypeptides, the invention further comprehends isolated and/or purified nucleic acid molecules and isolated and/or purified polypeptides having at least about 70%, preferably at least about 75% or about 77% identity or homology ("substantially homologous or identical"), advantageously at least about 80% or about 83%, such as at least about 85% or about 87% homolgy or identity ("significantly homologous or identical"), for instance at least about 90% or about 93% identity or homology ("highly homologous or identical"), more advantageously at least about 95%, e.g., at least about 97%, about 98%, about 99% or even about 100% identity or homology ("very highly homologous or identical" to "identical"; or from about 84-100% identity considered "highly conserved"). The invention also comprehends that these nucleic acid molecules and polypeptides can be used in the same fashion as the herein or aforementioned nucleic acid molecules and polypeptides.

Nucleotide sequence homology can be determined using the "Align" program of Myers and Miller, ("Optimal Alignments in Linear Space", CABIOS 4, 11-17, 1988, incorporated

herein by reference) and available at NCBI. Alternatively or additionally, the term “homology” or “identity”, for instance, with respect to a nucleotide or amino acid sequence, can indicate a quantitative measure of homology between two sequences. The percent sequence homology can be calculated as $(N_{ref} - N_{dif}) * 100 / N_{ref}$, wherein N_{dif} is the total number of non-identical residues in the two sequences when aligned and wherein N_{ref} is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence similarity of 75% with the sequence AATCAATC ($N_{ref} = 8$; $N_{dif} = 2$).

Alternatively or additionally, “homology” or “identity” with respect to sequences can refer to the number of positions with identical nucleotides or amino acids divided by the number of nucleotides or amino acids in the shorter of the two sequences wherein alignment of the two sequences can be determined in accordance with the Wilbur and Lipman algorithm (Wilbur and Lipman, 1983 PNAS USA 80:726, incorporated herein by reference), for instance, using a window size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4, and computer-assisted analysis and interpretation of the sequence data including alignment can be conveniently performed using commercially available programs (e.g., Intelligenetics™ Suite, Intelligenetics Inc. CA).. When RNA sequences are said to be similar, or have a degree of sequence identity or homology with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence.

RNA sequences within the scope of the invention can be derived from DNA sequences, by thymidine (T) in the DNA sequence being considered equal to uracil (U) in RNA sequences.

Additionally or alternatively, amino acid sequence similarity or identity or homology can be determined using the BlastP program (Altschul *et al.*, Nucl. Acids Res. 25, 3389-3402, incorporated herein by reference) and available at NCBI. The following references (each incorporated herein by reference) provide algorithms for comparing the relative identity or homology of amino acid residues of two proteins, and additionally or alternatively with respect to the foregoing, the teachings in these references can be used for determining percent homology or identity: Needleman SB and Wunsch CD, "A general method applicable to the search for similarities in the amino acid sequences of two proteins," J. Mol. Biol. 48:444-453 (1970); Smith TF and Waterman MS, "Comparison of Bio-sequences," Advances in Applied Mathematics 2:482-489 (1981); Smith TF, Waterman MS and Sadler JR, "Statistical characterization of nucleic acid sequence functional domains," Nucleic Acids Res., 11:2205-2220 (1983); Feng DF

and Doolittle RF, "Progressive sequence alignment as a prerequisite to correct phylogenetic trees," J. of Molec. Evol., 25:351-360 (1987); Higgins DG and Sharp PM, "Fast and sensitive multiple sequence alignment on a microcomputer," CABIOS, 5: 151-153 (1989); Thompson JD, Higgins DG and Gibson TJ, "ClusterW: improving the sensitivity of progressive multiple sequence alignment through sequence weighing, positions-specific gap penalties and weight matrix choice," Nucleic Acid Res., 22:4673-480 (1994); and, Devereux J, Haeberlie P and Smithies O, "A comprehensive set of sequence analysis program for the VAX," Nucl. Acids Res., 12: 387-395 (1984).

A polypeptide comprising at least a fragment or epitope of PspC, e.g., an epitopic region of PspC or PspC, can be a fusion protein; for instance, fused to a protein which enhances immunogenicity, such as a Cholera Toxin, e.g., Cholera Toxin B (CTB).

Similarly, a polypeptide comprising at least a fragment or epitope of PspC, e.g., an epitopic region of PspC or PspC, can be administered with an adjuvant or a vehicle which enhances immunogenicity, such as CTB.

Thus, the invention provides an immunological, immunogenic or vaccine composition comprising at least one PspC and/or a portion thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide comprising at least one epitope of PspA. The epitopic region of PspA can be as in applications cited under "Related Applications", *supra*, e.g., aa 1 to 115, 1 to 314, 1 to 260, 192 to 260, 192 to 588, 192 to 299, 1-301, 1-314 or 1-370 of PspA. From the teachings herein and in the applications cited under "Related Applications", the skilled artisan can select an epitope of interest, e.g., of PspC and/or PspA.

This invention also provides strain selection of PspCs from strains for vaccine compositions, based upon sequence homology and cross-reactivity, akin to that which Applicants have done with PspA. PspC strains can be classified according to sequence homology in the alpha helical and/or proline rich regions, and assigned to a clade, and subsequently, each clade is assigned to a family. Applicants have thus determined that so far there is at least one PspC family with at least two major clades.

Inventive compositions, such as immunogenic, immunological or vaccine compositions can comprise at least one PspC (or immunogenic fragment thereof or polypeptide comprising at least one PspC epitope or epitopic region or at least one vector or plasmid expressing such PspC or fragment thereof, or at least one PspC epitope or epitopic region), preferably at least two (2), for instance up to ten (10), from strains from each clade (and/or family), alone, or in further combination with at least one PspA (or immunogenic fragment thereof or polypeptide comprising at least one PspA or at least one epitope or epitopic region of PspA or at least one vector or plasmid expressing such PspA or fragment thereof, or at least one PspA epitope or epitopic region, which vector or plasmid can be the same as the aforementioned vector or plasmid) or preferably at least two (2), for instance up to ten (10), from strains from each PspA clade (and/or family), for a broadly efficacious pneumococcal vaccine with a limited number of strains.

Immunogenic, immunological or vaccine compositions of the invention can be administered in the same ways as PspA immunogenic, immunological or vaccine compositions, e.g., by injection, mucosally, orally, nasally, and the like, and/or by way of *in vivo* expression thereof by a plasmid or vector, as well as in same or similar regimens (e.g., such as by prime boost) (*see* applications cited under Related Applications, as well as documents cited herein). (Thus, there can be PspA, an epitopic region of PspA, a polypeptide comprising an epitope within an epitopic region of PspA, an immunogenic, immunological or vaccine composition comprising at least one PspA and/or at least one fragment or portion thereof, e.g., an epitopic region thereof or a polypeptide comprising at least one epitope from PspA and/or a vector or plasmid expressing a nucleic acid molecule encoding PspA or a fragment or portion thereof, administration of PspA or such a polypeptide or such a composition by injection, mucosally, nasally, orally, and the like and/or as part of a prime-boost regimen with another antigen which can also be PspA.) The amount of PspC in such compositions can be analogous to the amount of PspA in PspA immunogenic, immunological or vaccine compositions (*see* applications cited under Related Applications). (Accordingly, there can be PspC, an epitopic region of PspC, a polypeptide comprising an epitope within an epitopic region of PspC, an immunogenic, immunological or vaccine composition comprising at least one PspC and/or at least one fragment or portion thereof, e.g., an epitopic region thereof or a polypeptide comprising at least one epitope from PspC and/or a vector or plasmid expressing a nucleic acid molecule encoding PspC or a fragment or portion thereof, administration of PspC or such a polypeptide or such a

composition by injection, mucosally, nasally, orally, and the like and/or as part of a prime-boost regimen with another antigen which can also be PspC.)

Such compositions are useful in eliciting an immune response in an animal or a host, such as a protective immune response; or, for generating antibodies, which can be subsequently used in kits, tests or assays for detecting the presence of PspC and/or PspA and PspC and/or *S. pneumoniae*.

These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF FIGURES

The following Detailed Description, given by way of example, and not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

Fig. 1 shows a schmatic representation of the PspC clade A and clade B and PspA polypeptides in comparison with each other (long arrows represent direct repeats found within alpha helix; hypervariable region is indicated by zig-zag lines; and the region of homology of *pspC* with *pspA* found within the alpha helix is indicated by horizontal lines);

Fig. 2 shows the alignment of PspCs (SEQ ID NOS:) (the amino acid sequences which included the α helical region and the proline-rich region of PspC were aligned using MacVector 6.0; the direct repeats within the α helix, the non-coiled-coil block, and the proline-rich region are indicated with arrows; conserved regions are shaded, and gaps are shown with a dash (-); taxons are named for the strain from which the gene was cloned with the exception of Genbank entrees: SpsA1 (Y10818) from strain ATCC33400 (serotype 1), SpsA2 (AJ002054) from strain ATCC11733 (serotype 2),), SpsA47 (AJ002055) from strain NCTC10319 (serotype 47),), CbpA (AF019904) from strain LM91 (serotype 2), C3bp (AF067128), and tigr from a serotype 4 clinical isolate (<http://www.tigr.org>); the capsular serotypes of the other strains are as follows: EF6796 (6A), BG8090 (19), L81905 (4), DBL6A (6A), BG9163 (6B), D39 (2) and E134 (23));

Fig. 3 shows the coiled-coil motif of the alpha-helix of PspC (amino acids that are not in the coiled-coil motif are in the right column; this is the output from the Matcher program);

Fig. 4 shows a tree of the PspC proteins from this disclosure and related proteins SpsA and CbpA from Genbank (PspC proteins were truncated after the proline-rich region (Fig. 1) before being aligned using the ClustalW algorithm and the Blosum30 amino acid scoring matrix in MacVector; the tree is an unrooted phylogram generated by the neighbor-joining method using mean character distances in the program PAUP4.0b (Swofford); non-italic numbers on the tree indicate distances along the branch lengths as calculated by PAUP; italic bolded numbers indicate the percentage of time each branch was joined together under bootstrap analysis (1000 replicates performed); Clade A and Clade B are each monophyletic groups separated by greater than 0.1 distance which clustered together 100% of the time; Clade A PspC proteins share a 120 amino acid domain with many PspA proteins (Fig. 2); Clade B proteins lack the 120 AA domain, but all PspC/SpsA/CbpA proteins share the proline-rich domain with PspA proteins; the boxed D39-lineage indicates different sequences for this locus originating from strains that are laboratory descendents of the strain D39; the taxons used were the same as those described for Fig 2);

Fig. 5 shows PspC and PspA consensus of the choline binding region;

Fig. 6 shows the reactivity of the PspC antiserum with selected pneumococcal lysates run in a Western immunoblot ;

Fig. 7 shows the level of antibody reactivity to PspC and PspA fragments present in the sera of mice immunized with PspC (each bar represents the mean of the log reciprocal titer and upperbound of the standard error of sera from five mice; the limit of detection of the log reciprocal antibody titer is 1.8);

Fig. 8 shows amino acid and DNA sequences for SpsA and *spsA* from Genbank (SEQ ID NOS:) (accession CAA05158; AJ002054.1; AJ002054; Hammerschmidt et al. 1997);

Fig. 9 shows an additional amino acid and DNA sequences for SpsA and *spsA* from Genbank (SEQ ID NOS:) (accession CAA05159; AJ002055; AJ002055.1; Hammerschmidt et al. 1997);

Fig. 10 shows amino acid and DNA sequences for CbpA and *cbpA* from Genbank (SEQ ID NOS:) (accession AAB70838; AF019904; AF019904.1; Rosenow et al. 1997);

Fig. 11 shows amino acid and DNA sequences for PspC and *pspC* from Genbank (SEQ ID NOS:) (from EF6796; accession AAD00184; U72655.1; U72655; Brooks-Walter et al.);

Fig. 12 shows a tree of PspC proteins from this disclosure from the University of Alabama, analogous to the tree shown in Fig. 4 (PspC proteins sequenced at the University of Alabama; PspC proteins were truncated after the proline-rich region - *see* Fig. 1 - before aligned using the ClustalW algorithm and the Blosom30 amino acid scoring matrix in MacVector; the tree is an unrooted phylogram generated by the neighbor-joining method using mean character distances in the program PAUP4.0b (Swofford); non-italic numbers on the tree indicate distances along the branch lengths as calculated by PAUP; italic bolded numbers indicate the percentage of time each branch was joined together under bootstrap analysis (1000 replicates performed); Clade A and Clade B are monophyletic groups separated by greater than 0.1 distance which clustered together 100% of the time; Clade A PspC proteins share a 120 amino acid domain with many PspA protein - *see* Fig. 2; taxons are named for the strain from which the gene was cloned, with the capsular serotypes as follows - EF6796 (6A), BG8090 (19), L81905 (4), DBL6A (6a), BG9163 (6B), D39 (2) and E134 (23));

Fig. 13 shows the alignment of PspCs (SEQ ID NOS:) from this disclosure from the University of Alabama, analogous to the alignment shown in Fig. 2;

Fig. 14 shows a dendrogram showing the distance of a divergent PspC (from other PspCs), indicating that it likely belongs to a second family (Dendrogram of the PspC/SpsA/Cbpa from Genbank and nearest relative genes from other species; PspC proteins were truncated after the proline-rich region - *see* Fig. 1 - before being aligned using the ClustalW algorithm and the Blosom30 amino acid scoring matrix in MacVector; the dendrogram is the guide tree used in alignment by MacVector; small

numbers on the tree indicate distances along the branch lengths as calculated during the ClustalW alignment; sequences of two proteins from *Streptococcus agalactiae* bac and rib, and one from *Enterococcus faecalis* are included for comparison; the PspC.V26 is a highly divergent PspC protein from *S. pneumoniae* strain V26);

Fig. 15 shows the amino acid and DNA sequences (SEQ ID NOS:) of the divergent PspC (PspC from *S. pneumoniae* strain V26);

Figs. 16-21 show the DNA sequences (SEQ ID NOS:) of PspCs from strains E134, D39, BG9163, BG8090, L81905, and DBL6a, respectively.

DETAILED DESCRIPTION

PspC (see Figs. 1, 2, 3, 4, 5, 11, 12, 13, 14, 15) is one of three designations for a pneumococcal surface protein which is PspA-like, and whose gene is present in approximately 75% of all *Streptococcus pneumoniae*. Applicants have cloned and sequenced the *pspC* gene and have expressed the PspC protein (See, e.g., Figs. 1, 2, 4, 5, 11, 12, 13, and patent applications cited under the heading Related Applications, *supra*, as well as to articles or literature cited herein; see also Figs. 14, 15). Under the designation SpsA (see Figs. 8, 9), PspC has been shown to bind secretory IgA (Hammerschmidt et al. 1997). Under the designation CbpA (see Fig. 10), PspC has been shown to interact with human epithelial and endothelial cells (Rosenow et al. 1997).

The *pspC* gene is paralogous to the *pspA* gene in *S. pneumoniae* and was thus called *pspC* (Brooks-Walter et al. 1997; see also applications cited in Related Applications, *supra*).

The present invention provides epitopic regions of PspC, different clades of PspC, DNA encoding epitopic regions of PspC, vectors which express such epitopic regions, immunological, immunogenic or vaccine compositions comprising at least one PspC and/or a portion thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide comprising at least one epitope of PspA.

PspC or a fragment thereof, and thus a composition comprising PspC or a fragment thereof, can be administered by the same routes, and in approximately the same amounts, as PspA. Thus, the invention further provides methods for administering PspC or a fragment

thereof or a polypeptide comprising at least one epitope of PspC, as well as uses of PspC or a fragment thereof to formulate such compositions.

Furthermore, in this disclosure, *pspC* genes from seven different clinical *S. pneumoniae* strains were cloned and sequenced. Examination of the sequences of twelve alleles reveals that this gene exists in diverse forms among pneumococci and has a mosaic structure in which sequence modules encoding protein domains have contributed to the pattern of variation during gene evolution.

Two major clades exist: clade A alleles are larger and contain an extra module that is shared by many *pspA* genes; clade B alleles are smaller and lack this *pspA*-like domain. All genes in both clade A and clade B maintain a proline-rich domain and a choline-binding repeat domain that are indistinguishable from similar domains in the *pspA* gene at the nucleotide and protein level.

Thus, this invention also relates to strain selection of PspCs from strains for vaccine compositions, based upon sequence homology and cross-reactivity, akin to that which Applicants have done with PspA. PspC strains can be classified according to sequence homology in the alpha helical and/or proline rich regions, and assigned to a clade, and subsequently, each clade is assigned to a family. Applicants have thus determined that so far there is one PspC family with at least two major clades.

There is, however, a single PspC (PspC.V26, from *S. pneumoniae* strain V26, a capsular-type 14 *S. pneumoniae* strain) that appears to be a member of a second family because it seems only distantly related to members of the first major PspC family. Figure 14 provides a dendrogram showing the distance of this divergent PspC from the other PspCs. Fig. 15 provides the amino acid and DNA sequences of the divergent PspC.

Inventive compositions, such as immunogenic, immunological or vaccine compositions can comprise at least one PspC (or immunogenic fragment thereof or polypeptide comprising at least one PspC epitope or epitopic region or at least one vector or plasmid expressing such PspC or fragment thereof, or at least one PspC epitope or epitopic region), preferably at least two (2), for instance up to ten (10), from strains from each clade, alone, or in further combination with at least one PspA (or immunogenic fragment thereof or polypeptide comprising at least one PspA or at least one epitope or epitopic region of PspA or at least one vector or plasmid expressing such PspA or fragment thereof, or at least one PspA epitope or epitopic region, which vector or

plasmid can be the same as the aforementioned vector or plasmid) or preferably at least two (2), for instance up to ten (10), from strains from each PspA clade, for a broadly efficacious pneumococcal vaccine with a limited number of strains.

Accordingly, in an aspect, the invention provides an immunogenic, immunological or vaccine composition containing an epitope of interest from at least one PspC and/or PspA, and a pharmaceutically acceptable carrier or diluent. An immunological composition elicits an immunological response - local or systemic. The response can, but need not be, protective. An immunogenic composition likewise elicits a local or systemic immunological response which can, but need not be, protective. A vaccine composition elicits a local or systemic protective response. Accordingly, the terms "immunological composition" and "immunogenic composition" include a "vaccine composition" (as the two former terms can be protective compositions).

The invention therefore also provides a method of inducing an immunological response in a host mammal comprising administering to the host an immunogenic, immunological or vaccine composition. From the disclosure herein and the documents cited herein, including the applications cited under "Related Applications", the skilled artisan can obtain an epitope of interest of PspC and/or PspA, without undue experimentation.

Further, the invention demonstrates that more than one serologically complementary PspC molecule can be in an antigenic, immunological or vaccine composition, so as to elicit better response, e.g., protection, for instance, against a variety of strains of pneumococci; and, the invention provides a system of selecting PspCs for a multivalent composition which includes cross-protection evaluation so as to provide a maximally efficacious composition.

The determination of the amount of antigen, e.g., PspC or truncated portion thereof or a polypeptide comprising an epitope or epitopic region of PspC, and optional adjuvant in the inventive compositions and the preparation of those compositions can be in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary arts.

In particular, the amount of antigen and adjuvant in the inventive compositions and the dosages administered are determined by techniques well known to those skilled in the medical or veterinary arts taking into consideration such factors as the particular antigen, the adjuvant (if present), the age, sex, weight, species and condition of the particular patient, and the route of administration.

For instance, dosages of particular PspC antigens for suitable hosts in which an immunological response is desired, can be readily ascertained by those skilled in the art from this disclosure, as is the amount of any adjuvant typically administered therewith. Thus, the skilled artisan can readily determine the amount of antigen and optional adjuvant in compositions and to be administered in methods of the invention. Typically, an adjuvant is commonly used as 0.001 to 50 wt% solution in phosphate buffered saline, and the antigen is present on the order of micrograms to milligrams, such as about 0.0001 to about 5 wt%, preferably about 0.0001 to about 1 wt%, most preferably about 0.0001 to about 0.05 wt% (see, e.g., Examples below or in applications cited herein).

Typically, however, the antigen is present in an amount on the order of micrograms to milligrams, or, about 0.001 to about 20 wt%, preferably about 0.01 to about 10 wt%, and most preferably about 0.05 to about 5 wt%.

Of course, for any composition to be administered to an animal or human, including the components thereof, and for any particular method of administration, it is preferred to determine therefor: toxicity, such as by determining the lethal dose (LD) and LD₅₀ in a suitable animal model e.g., rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable immunological response, such as by titrations of sera and analysis thereof for antibodies or antigens, e.g., by ELISA and/or RFFIT analysis. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, vaginal, peroral, intragastric, mucosal (e.g., perlingual, alveolar, gingival, olfactory or respiratory mucosa) etc., administration such as suspensions, syrups or elixirs; and, preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors,

and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Compositions of the invention, are conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions or viscous compositions which may be buffered to a selected pH. If digestive tract absorption is preferred, compositions of the invention can be in the "solid" form of pills, tablets, capsules, caplets and the like, including "solid" preparations which are time-released or which have a liquid filling, e.g., gelatin covered liquid, whereby the gelatin is dissolved in the stomach for delivery to the gut. If nasal or respiratory (mucosal) administration is desired, compositions may be in a form and dispensed by a squeeze spray dispenser, pump dispenser or aerosol dispenser. Aerosols are usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or, a dose having a particular particle size.

Compositions of the invention can contain pharmaceutically acceptable flavors and/or colors for rendering them more appealing, especially if they are administered orally. The viscous compositions may be in the form of gels, lotions, ointments, creams and the like and will typically contain a sufficient amount of a thickening agent so that the viscosity is from about 2500 to 6500 cps, although more viscous compositions, even up to 10,000 cps may be employed. Viscous compositions have a viscosity preferably of 2500 to 5000 cps, since above that range they become more difficult to administer. However, above that range, the compositions can approach solid or gelatin forms which are then easily administered as a swallowed pill for oral ingestion.

Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection or orally, to animals, children, particularly small children, and others who may have difficulty swallowing a pill, tablet, capsule or the like, or in multi-dose situations. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with mucosa, such as the lining of the stomach or nasal mucosa.

Obviously, the choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form), or solid dosage form (e.g., whether the composition is to be formulated into a pill, tablet, capsule, caplet, time release form or liquid-filled form).

Solutions, suspensions and gels, normally contain a major amount of water (preferably purified water) in addition to the antigen, lipoprotein and optional adjuvant. Minor amounts of other ingredients such as pH adjusters (e.g., a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservatives, wetting agents, jelling agents, (e.g., methylcellulose), colors and/or flavors may also be present. The compositions can be isotonic, i.e., it can have the same osmotic pressure as blood and lacrimal fluid.

The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

Viscosity of the compositions may be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount which will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

A pharmaceutically acceptable preservative can be employed to increase the shelf-life of the compositions. Benzyl alcohol may be suitable, although a variety of preservatives including, for example, parabens, thimerosal, chlorobutanol, or benzalkonium chloride may also be employed. A suitable concentration of the preservative will be from 0.02% to 2% based on the total weight although there may be appreciable variation depending upon the agent selected.

Those skilled in the art will recognize that the components of the compositions must be selected to be chemically inert with respect to the PspC antigen and optional adjuvant. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can

be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

The immunologically effective compositions of this invention are prepared by mixing the ingredients following generally accepted procedures. For example the selected components may be simply mixed in a blender, or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity. Generally the pH may be from about 3 to 7.5. Compositions can be administered in dosages and by techniques well known to those skilled in the medical and veterinary arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient or animal, and the composition form used for administration (e.g., solid vs. liquid). Dosages for humans or other mammals can be determined without undue experimentation by the skilled artisan, from this disclosure, the documents cited herein, the Examples below (e.g., from the Examples involving mice and from the applications cited herein, e.g., under "Related Applications", especially since PspC can be administered in a manner and dose analogous to PspA).

Suitable regimes for initial administration and booster doses or for sequential administrations also are variable, may include an initial administration followed by subsequent administrations; but nonetheless, may be ascertained by the skilled artisan, from this disclosure, the documents cited herein, including applications cited herein, and the Examples below. The compositions can be administered alone, or can be co-administered or sequentially administered with other compositions of the invention or with other prophylactic or therapeutic compositions. Given that PspC is PspA-like, the skilled artisan can readily adjust concentrations of PspA in compositions comprising PspA or a portion thereof to take into account the presence of PspC or a portion thereof in accordance with the herein teachings of compositions comprising at least one PspC or portion thereof and optionally at least one PspA or a portion thereof.

The PspC antigen (PspC or a portion thereof), as well as a PspA antigen (PspA or a portion thereof) can be expressed recombinantly, e.g., in *E. coli* or in another vector or plasmid for either *in vivo* expression or *in vitro* expression. The methods for making and/or administering a vector or recombinant or plasmid for expression of PspC or a portion thereof either *in vivo* or *in vitro* can be any desired method, e.g., a method which is by or analogous to

the methods disclosed in: U.S. Patent Nos. 4,603,112, 4,769,330, 5,174,993, 5,505,941, 5,338,683, 5,494,807, 4,722,848, WO 94/16716, WO 96/39491, Paoletti, "Applications of pox virus vectors to vaccination: An update," PNAS USA 93:11349-11353, October 1996, Moss, "Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety," PNAS USA 93:11341-11348, October 1996, Smith et al., U.S. Patent No. 4,745,051 (recombinant baculovirus), Richardson, C.D. (Editor), Methods in Molecular Biology 39, "Baculovirus Expression Protocols" (1995 Humana Press Inc.), Smith et al., "Production of Huma Beta Interferon in Insect Cells Infected with a Baculovirus Expression Vector," Molecular and Cellular Biology, Dec., 1983, Vol. 3, No. 12, p. 2156-2165; Pennock et al., "Strong and Regulated Expression of *Escherichia coli* B-Galactosidase in Infect Cells with a Baculovirus vector," Molecular and Cellular Biology Mar. 1984, Vol. 4, No. 3, p. 399-406; EPA 0 370 573, U.S. application Serial No. 920,197, filed October 16, 1986, EP Patent publication No. 265785, U.S. Patent No. 4,769,331 (recombinant herpesvirus), Roizman, "The function of herpes simplex virus genes: A primer for genetic engineering of novel vectors," PNAS USA 93:11307-11312, October 1996, Andreansky et al., "The application of genetically engineered herpes simplex viruses to the treatment of experimental brain tumors," PNAS USA 93:11313-11318, October 1996, Robertson et al. "Epstein-Barr virus vectors for gene delivery to B lymphocytes," PNAS USA 93:11334-11340, October 1996, Frolov et al., "Alphavirus-based expression vectors: Strategies and applications," PNAS USA 93:11371-11377, October 1996, Kitson et al., J. Virol. 65, 3068-3075, 1991; U.S. Patent Nos. 5,591,439, 5,552,143 (recombinant adenovirus), Grunhaus et al., 1992, "Adenovirus as cloning vectors," Seminars in Virology (Vol. 3) p. 237-52, 1993, Ballay et al. EMBO Journal, vol. 4, p. 3861-65, Graham, Tibtech 8, 85-87, April, 1990, Prevec et al., J. Gen Virol. 70, 429-434, PCT WO91/11525, Felgner et al. (1994), J. Biol. Chem. 269, 2550-2561, Science, 259:1745-49, 1993 and McClements et al., "Immunization with DNA vaccines encoding glycoprotein D or glycoprotein B, alone or in combination, induces protective immunity in animal models of herpes simplex virus-2 disease," PNAS USA 93:11414-11420, October 1996, and U.S. Patents Nos 5,591,639, 5,589,466, and 5,580,859 relating to DNA expression vectors, *inter alia*. See also WO 98/33510; Ju et al., Diabetologia, 41:736-739, 1998 (lentiviral expression system); Sanford et al., U.S. Patent No. 4,945,050 (method for transporting substances into living cells and tissues and apparatus therefor); Fischbach et al. (Intracel), WO 90/01543 (method for the genetic expression of heterologous proteins by cells transfected);

Robinson et al., seminars in IMMUNOLOGY, vol. 9, pp.271-283 (1997) (DNA vaccines); Szoka et al., U.S. Patent No. 4,394,448 (method of inserting DNA into living cells); and McCormick et al., U.S. Patent No. 5,677,178 (use of cytopathic viruses for therapy and prophylaxis of neoplasia).

The expression product generated by vectors or recombinants in this invention optionally can also be isolated and/or purified from infected or transfected cells; for instance, to prepare compositions for administration to patients. However, in certain instances, it may be advantageous to not isolate and/or purify an expression product from a cell; for instance, when the cell or portions thereof enhance the effect of the polypeptide.

An inventive vector or recombinant expressing PspC or a portion thereof and/or PspA or a portion thereof can be administered in any suitable amount to achieve expression at a suitable dosage level, e.g., a dosage level analogous to the aforementioned dosage levels (wherein the antigen or epitope of interest is directly present). The inventive vector or recombinant can be administered to a patient or infected or transfected into cells in an amount of about at least 10^3 pfu; more preferably about 10^4 pfu to about 10^{10} pfu, e.g., about 10^5 pfu to about 10^9 pfu, for instance about 10^6 pfu to about 10^8 pfu. In plasmid compositions, the dosage should be a sufficient amount of plasmid to elicit a response analogous to compositions wherein PspC or a portion thereof and/or PspA or a portion thereof are directly present; or to have expression analogous to dosages in such compositions; or to have expression analogous to expression obtained *in vivo* by recombinant compositions. For instance, suitable quantities of plasmid DNA in plasmid compositions can be 1 μ g to 100 mg, preferably 0.1 to 10 mg, e.g., 500 micrograms, but lower levels such as 0.1 to 2 mg or preferably 1-10 μ g may be employed. Documents cited herein regarding DNA plasmid vectors may be consulted for the skilled artisan to ascertain other suitable dosages for DNA plasmid vector compositions of the invention, without undue experimentation.

Returning to our discussion of the examples and results presented herein, a rabbit polyclonal serum to PspC was made by immunization with a recombinant truncated clade B allele. The serum reacted with both PspC and PspA from fifteen (15) pneumococcal isolates indicating that PspC and PspA share extensive cross-reactive epitopes. The cross-reactive antibodies appeared to cause cross-protection in a mouse model system. Mice immunized with recombinant clade B PspC were protected against challenge with a strain that expressed PspA

but not PspC. In this experiment, the PspA-PspC cross-reactive antibodies were directed to the proline-rich domain present in both molecules.

More in particular, *S. pneumoniae* possess a family of proteins that bind phosphocholine (Brooks-Walter et al. 1997; Garcia et al. 1986; McDaniel et al. 1992) present in the teichoic acid and the lipoteichoic acid of the cell membrane and the cell wall (Tomasz 1967). The choline-binding proteins of pneumococci and other gram-positive organisms all contain structurally similar choline-binding domains, which are composed of multiple tandem amino acid repeats (Breise et al. 1985). Autolysin, PspA (pneumococcal surface protein A), and PcpA (pneumococcal choline-binding protein A) of *S. pneumoniae*, toxins A and B of *Clostridium difficile*, glucosyltransferases from *Streptococcus downei* and *Streptococcus mutans*, CspA of *Clostridium acetobutylicum*, and PspA of *Clostridium perfringens* all contain similar regions (Sanchez-Beato et al. 1995; Banas et al. 1990; Barroso et al. 1990; Dove et al. 1990; Garcia et al. 1986; Sanchez-Beato et al. 1998).

In PspA from *S. pneumoniae*, these choline-binding repeats are responsible for the attachment of PspA to the surface of the pneumococcus (Yother et al. 1994). PspA molecules interfere with complement activation (Briles et al. 1997), slow clearance of pneumococci from the blood of infected mice (McDaniel et al. 1987), and elicit protection against pneumococcal sepsis and nasal carriage (McDaniel et al. 1991; Wu et al. 1997). A single non-*pspA* locus has been identified which has greater similarity to the choline-binding and proline rich regions of *pspA* than any of the other choline-binding genes (McDaniel et al. 1992). Applicants have designated the molecule PspC because of its strong molecular and serologic similarities to PspA (Brooks-Walter et al. 1997; *see also* applications cited under Related Applications, *supra*, note that in those applications initially PspC was called "PspA-like", and *pspC* was considered *pspA*-like).

Other PspA-like proteins and *pspA*-like loci, which could be the same as PspC and *pspC*, have also been characterized and sequenced (SpsA, which reportedly binds secretory IgA, Hammerschmidt et al. 1997; choline-binding protein (for binding a moiety on eukaryotic surfaces), CbpA, Rosenow et al. 1997; *see, e.g.*, Figs. 8, 9, 10).. Immunization with a crude extract of pooled non-PspA choline-binding proteins containing CbpA elicited protection to a lethal challenge of pneumococci introduced intraperitoneally into mice (Rosenow et al. 1997).

In the present studies, Applicants have demonstrated that immunization with purified PspC is able to elicit protection against sepsis, and this protection is apparently mediated by antibodies cross-reactive with PspA. Applicants have also examined the genetic diversity present within this genetic locus, herein called *pspC*, by the examination of 12 sequenced alleles. These include the previously sequenced alleles of *cbpA* and *spsA*, an allele from the genomic sequencing project, and seven newly sequenced *pspC* genes presented here for the first time.

The sequences of *cbpA* and *spsA* both included sequences of D39 or its derivatives. Rosenow et al. sequenced *cbpA* from LM91 a *pspA*- mutant of D39 (Rosenow et al. 1997); and Hammerschmidt et al. sequenced *spsA* from an encapsulated derivative of R36A (ATCC11733) (Hammerschmidt et al. 1997; *see also* Figs. 8, 9, 10). From a comparison of these two sequences, it was apparent that *spsA* sequence contained a 480 bp deletion within the gene. Because of this discrepancy, Applicants also reported a sequence of *pspC* from a cloned *HindIII*-*EcoRI* chromosomal fragment of D39 that was determined prior to the *cbpA* and *spsA* sequence (Brooks-Walter et al. 1997; *see also* applications cited under Related Applications, *supra*). This sequence matched exactly that of *cbpA*. Other sequences that were used for sequence alignment comparisons included two *spsA* sequences from capsular serotype 1 and 47 strains (Hammerschmidt et al. 1997), and the *pspC/cbpA/spsA* sequence from the capsular serotype 4 strain sequenced in the TIGR genome project (accessed by the internet at <http://www.tigr.org>).

The invention shall be further described by way of the following Examples And Results, provided for illustration and not to be considered a limitation of the invention.

EXAMPLES AND RESULTS

Materials and methods

Bacterial strains, plasmids, and recombinant DNA techniques

Chromosomal DNA from *S. pneumoniae* EF6796, a serotype 6A clinical isolate (Salser et al. 1993) and D39, a serotype 2 isolate, was isolated using a cesium chloride gradient procedure. The *HindIII*-*EcoRI* fragment of EF6796 and D39 was cloned in a modified pZero vector (Invitrogen, San Diego, CA) in which the Zeocin-resistance cassette was replaced by a kanamycin cassette, kindly provided by Randall Harris. Recombinant plasmids were electroporated into *Escherichia coli* TOP10F' cells [F' {*lacI*^qTet^R} *mcrA*_(*mrr-hsdRMS-mcrBC*) f80*lacZ*_M15 _*lacX74 deoR recA1 araD139* _ (*ara-leu*)7697 *galU galK rpsL endA1*

nupG] (Invitrogen). DNA was purified from agarose using Gene Clean (Bio101, Inc., Vista, CA).

Chromosomal DNA used for PCR was isolated using a chloroform-isoamyl alcohol procedure. Oligonucleotide primers, ABW13 (5' CGACGAATAGCTGAAGAGG 3') (SEQ ID NO:) and SKH2 (5'CATACCGTTTTCTTGTTTCCAGCC 3') (SEQ ID NO:), were used to amplify the DNA encoding the alpha helical region and the proline rich region of *pspC* in 100 additional *S. pneumoniae* strains. These primers correspond to nucleotides 215-235 and nucleotides 1810-1834, respectively, of the *pspC*/EF6796 gene. PCR products from L81905 (serotype 4), BG9163 (serotype 6B), DBL6A (serotype 6A), BG8090 (serotype 19) and E134 (serotype 23) were cloned into pGem (Promega) or Topo TA vector (Invitrogen) which utilize the A over hangs generated by Taq polymerase.

Sequencing and DNA analysis

Sequencing of *pspC* was completed using automated DNA sequencing (ABI 377, Applied Biosystems, Inc., Foster City, CA). Sequence analyses were performed using the University of Wisconsin Genetics Computer Group (GCG) programs (Devereux et al. 1984), MacVector 6.5 (Oxford Molecular), Sequencer 3.0 (GeneCodes, Inc.), and DNA Strider programs (Salser et al. 1993). Sequence similarities of *pspC* were determined using the NCBI BLAST -coil structure predicted by *pspC* sequence was analyzed using Matcher (Fischetti et al. 1993). The accession number by Genbank/EMBL for the nucleotide sequence of PspC are as follows: EF6796 -U72655, DBL6A- AF068645, D39-AF068646, E134 -AF068647, BG8090-AF068648, L81905 - AF068649, BG9163 -AF068650, DBL6A - AF068645, D39 - AF068646, E134 - AF068647, BG8090 - AF068648, L81905 - AF068649, and BG9163 - AF068650; and each of these sequences and GenBank results from the accession numbers are hereby expressly incorporated herein by reference (*See also* Figs. 11 and 15-21) . Preliminary sequence data was obtained from The Institute for Genomic Research website at [http:// www.tigr.org](http://www.tigr.org).

Example/Result 1: Sequence analysis of *pspC* gene – aspects relating to domain structure and function:

The protein sequences of *pspC*, *spsA*, and *cbpA* were aligned using MacVector 6.5 (Figures 1, 2, and 13). The predicted amino acid sequences encode proteins ranging in size from 59 to 105 kDa protein. The signal sequences of 37 amino acids are highly conserved (84-100% Identity). The major part of each protein is composed of a large alpha-helical domain (Figures 1,

2, and 13). The N-terminal 100 – 150 amino acids of this alpha-helical domain are hypervariable in both size and sequence and are unique for each strain sequenced of unrelated parentage (Figure 2, D39, SpsA2, CbpA, and Cb3P are all from a related lineage; *see also* Fig. 13). In the hypervariable regions of capsular serotype 1 and 4 strains, there is a unique 23 amino acid serine-rich sequence (amino acid positions 112 to 135).

Downstream of the hypervariable region and central to the alpha-helical domain is the first of two direct repeats. The amino acid repeats (Figures 2, 13) vary in size in individual PspCs from 101 to 205 amino acids and are approximately 79-89% identical at the amino acid level. Smaller-sized amino acid repeats in some strains differ from the larger repeats of other strains only by lack of sequence at the NH₂-terminal end, which accounts for their smaller size. The first repeat in each strain is more like the corresponding first repeat of other strains than it is like the second repeat of the same strain. This pattern suggests that duplication forming this repeat happened in an ancestral gene, prior to the diversification of *pspC* into the numerous divergent alleles seen today. These repeats are highly charged with approximately 45% of their sequence being either lysine or glutamic acid residues. These alpha-helical repeats were present in all alleles that were examined except for the *spsA*//serotype 1 and *spsA*//serotype 2 (Hammerschmidt et al. 1997) (Figures 2, 13).

Between the amino acid repeats of the alpha-helical domain is a highly conserved 40 amino acid sequence break in the coiled-coil motif which was identified using the Matcher program (Fischetti et al. 1993) (Figures 2, 13 and 3). Matcher examines the characteristic seven residue periodicity of coiled-coil proteins arising largely from the predominance of hydrophobic residues in the first and fourth positions (a and d) and non-hydrophobic residues in the remaining positions (Fischetti et al. 1993). The coiled-coil region of the alpha-helix of PspC/EF6796 has three breaks in the heptad repeat motif (Figure 3). These interruptions of the heptad motif in the 7-residue periodicity were respectively 6, 44 and 5 amino acids in length. Similar breaks at corresponding sequence positions were found in all PspC alleles.

In some molecules of PspC, the proline-rich region followed the second amino acid repeat (Figures 1, 2, and 13). However, in the three larger PspC molecules, a region very similar to a corresponding region of the *pspA* genetic locus is present. Based on whether this *pspA*-like region was present or absent and on a distance-based cluster analysis, PspC molecules were classified into two clades (Figure 4, 12). Clade A molecules contained the *pspA*-like element

and were larger in size. PspC clade B molecules were smaller and lacked this *pspA*-like region. This *pspA*-like region (alpha-helical-2) was present in PspC/BG9163, EF6796 and BG7322 (Figures 1, 2, and 13 Table 1) as well as in many *pspA* genes.

Although there is some variation within the proline-rich region of the sequenced PspCs (Figures 1, 2, 13), the region is not distinguishable from the proline-rich region of PspA molecules. Within PspA molecules, two types of proline-rich regions have been identified. One type, which corresponds to about 60% of PspAs, contains a central region of 27 non-proline amino acids, which is highly conserved. The other type of proline-rich region in PspA lacks this conserved non-proline region. In the case of PspC, clade A strains lacked the 27 amino acid non-proline-rich block, whereas the four clade B PspC molecules had this conserved block. When present, the sequence of the 27 amino acid non-proline-rich region is highly conserved between PspC and PspA molecules. No correlation was observed between the expression of this conserved region within PspA and PspC molecules produced by the same strain. The proline-rich region of SpsA/serotype 1 was different from that of all other PspC molecules. This proline-rich region of this SpsA molecule has a truncated proline-rich region, which contains the 27 amino acid non-proline break but lacks the NH₂ end of the proline-rich region.

The choline-binding repeat domains of PspC, CbpA and SpsA proteins each contain between 4 and 11 repeats of about 20 amino acids (Figure 5). The repeats found in the center of the choline-binding domain were closest to the consensus sequence, while repeats on the NH₂-terminal and COOH-terminal ends of the block were more distant from the consensus sequence. The arrangement of repeats over the entire choline-binding region in PspC was examined relative to the arrangement of similar repeats in the choline-binding region of five PspC and three PspA genes for which the entire choline-binding domain was sequenced. The following findings all suggested a very close relationship between PspA and PspC in the choline-binding region of the molecule: 1) the NH₂-terminal divergent repeat is identical between the paralogous proteins (PspA and PspC); 2) similarly, the COOH-terminal divergent repeats are very similar between PspC and PspA (see repeats 10 and 11 of PspC consensus and repeats 9 and 10 of PspA consensus – Figure 5), yet these repeats are highly diverged from the rest of the repeat block; 3) the conserved central repeats of the choline-binding domain in each case have a single amino acid at position 6 which is frequently asparagine in PspC, but usually tyrosine in PspA proteins. Other than position 6, the consensus repeat for both genes is identical;

4) Divergence of individual amino acids within the 20 amino acid repeat from the repeat consensus sequence was identical between PspA and PspC (position number 4,6,9,12,13,15,16, and 18); and 5) The repeat blocks are followed by a 17 amino acid partially hydrophobic “tail” that is nearly identical for PspC or PspA except for an additional asparagine present at the end of the PspC proteins that is missing from PspA proteins. Overall, the choline-binding domains of PspA and PspC are so similar that it would not be possible to determine with certainty whether any particular choline-binding domain from these two proteins belongs to PspA or PspC without knowledge of its flanking DNA.

Example/Result 2: Phylogenetic Analysis:

The *pspA* and *pspC* genes are paralogs of each other because they are both present in the genome of most pneumococci, and because they share high identity in the sequence encoding their COOH-terminal halves (Table 1). An alignment of the 12 PspC/CbpA/SpsA sequences was constructed using the Clustal W algorithm (Figures 2, 13). An unrooted phylogram was produced with PAUP 4.0B with the neighbor-joining method from the mean amino acid distances as calculated over this alignment (Figures 4, 12). The figure as shown incorporates both distance measurements along the branch lengths and bootstrap analysis of 1000 repetitions. Branch length between molecules is proportional to the similarity of the sequences. The tree represents the evolutionary hypothesis that PspC molecules arose in two main clusters representing clades A and B. One clade, A, consisted of the larger PspC molecules, and contained strong identity in alpha-helical region-2 with some *pspA* alleles. The second clade, B, did not contain this region of identity with *pspA* alpha-helical region *pspAs*.

Example/Result 3: Analysis of *pspC* using PCR:

PCR was used to amplify *pspC* from different strains of *S. pneumoniae* to permit studies of the variability of PspC. Two oligonucleotides which recognized the common sequence regions of *pspC*, but which did not amplify the *pspA* genes, were designed in an effort to permit specific amplification of *pspC* alleles from all pneumococcal strains. Oligonucleotide ABW13 is specific to DNA upstream of the promoter sequence of the *pspC* gene locus. Oligonucleotide SKH2 is specific to the DNA encoding the C-terminal end of the proline-rich region of both the *pspA* and *pspC* gene loci. These oligonucleotides were used to amplify fragments of *pspC* from 100 *S. pneumoniae* strains. Seventy-eight of the 100 strains produced PCR-generated fragments, which varied from 1.5 kb to 2.2 kb in size. The remaining 22 strains failed to produce a PCR

product. Based on the strains of known sequence it was observed that the size of the amplified products correlated with whether they were clade A or clade B. Because of the absence of this *pspA*-conserved region, the clade B *pspC* sequences were smaller than the clade A *pspC*. The amplified product using oligonucleotide ABW13 and SKH2 of clade A molecules was 2.0 kb or greater. The amplified fragment of clade B molecules was approximately 1.6 kb. Approximately 4% of the 75 strains from which a *pspC* gene was amplified were found to be clade A by this criterion and 96% were clade B.

Example/Result 4: Cloning and expression of a recombinant truncated PspC molecules:

Oligonucleotides were used to amplify a 1.2 kb fragment of L81905, which encodes amino acids 263-482 of the alpha-helix and proline-rich region of PspC. The amplified PCR fragment was cloned into pQE40 (Qiagen, Chatsworth, CA) which allows expression of a fusion product with a polyhistidine tag at the amino-terminal end, followed by dihydrofolate reductase (DHFR), and then by the fragment of PspC/L81905 (263-482). Expression of the fusion protein in *Escherichia coli* strain BL21(DE3) was induced during growth at room temperature by the addition of 1 mM isopropyl-*b*-d-thiogalactopyranoside (IPTG). The overexpressed fusion protein was purified by affinity chromatography under non-denaturing conditions over a nickel resin according to the manufacturer's protocols. Purified fusion protein was then analyzed by SDS-PAGE and quantitated using a BioRad Protein Assay (Hercules, CA). Two fragments of PspC/D39 (AA 1-445 and AA 255-445), and three fragment of PspA/Rx1 (AA 1-301, AA 1-314 and AA 1-370) were expressed as fusion proteins with 6X His tag in the pET20b expression system (Novagen, Madison, WI). In this case, the overexpressed fusion proteins contain a PelB leader peptide, followed by the PspC or PspA fragments and the His tag at the carboxy-terminus. Expression was induced for pET20b-based constructs with 0.4mM IPTG in the expression strain BL21(DE3), and purified according the manufacturer's protocol.

Example/Result 5: Production of a polyclonal antiserum, SDS-PAGE, and immunoblots:

The truncated product (AA 263 to 482) of PspC/L81905 was purified by metal affinity chromatography and used to immunize a rabbit. Approximately 4 µg of purified PspC from L81905 was injected two times subcutaneously into a rabbit twice on consecutive weeks and blood was collected 10 days after the last injection. The primary immunization was with Freund's complete adjuvant and the booster immunization was given in saline. Polyclonal rabbit antiserum was diluted 1:50 and used to analyze pneumococcal lysates on a 7.5% SDS-PAGE gel

(BioRad, Hercules, CA). Pneumococcal lysates and immunoblots were performed as described by Yother et al. 1994.

**Example/Result 6: Cross-reactivity of antisera made to
PspC/L81905 with PspA and other PspC molecules:**

A truncated product (AA 263-482) of the PspC/L81905 clade B pspC protein was expressed in *E. coli* using the Qiagen Expression system. It should be noted that PspC/L81905 is clade B and lacks the *pspA*-like region in its alpha-helix. The truncated (AA263-482) clade B PspC protein was purified by metal affinity chromatography and used to immunize a rabbit to generate a polyclonal antiserum to PspC. Pneumococcal lysates were separated on SDS-polyacrylamide gels and blotted to nitrocellulose. The blots were developed either with Xi126, a monoclonal antibody to PspA, or with the anti-PspC rabbit polyclonal antiserum. The reactivity of the PspC antiserum with selected pneumococcal lysates run in a Western immunoblot is shown in Figure 6.

The reactivity pattern of the antiserum to PspC was deciphered in part using lysates from *S. pneumoniae* strains JY1119 and JY53. These strains are derivatives of the pneumococcal strains WU2 and D39, respectively, in which the *pspA* genes have been insertionally inactivated (Yother et al 1992). From the Western blot, it is apparent that the polyclonal serum reacts with a 90 kDa band in JY53 even though the *pspA* gene has been inactivated in this strain. This band is assumed to represent PspC. Both JY1119 and its parent, WU2, lack the *pspC* gene altogether (McDaniel et al. 1992). An 85 kDa molecule from WU2 reacts with the anti-PspC antiserum and with the anti-PspA MAb. This band is not present in JY1119, which contains an insertionally inactivated PspA.

The rabbit antiserum was reactive with proteins in the lysates from all pneumococcal strains tested. The relative molecular weights of the proteins detected also made it apparent that the antiserum was reacting with both PspA and PspC molecules. To distinguish cross-reactivity with the PspA molecule from direct reactivity with the PspC molecule in untested strain lysates a second identical Western blot was developed with a monoclonal antibody specific to PspA molecules (Figure 6, part B). PspC bands could be identified through the comparison of banding patterns in parts A and B of Figure 6. The bands reactive the anti-PspC rabbit antiserum but not with the anti-PspA MAb were identified as PspC. Bands stained by the rabbit antiserum that co-migrate with those also stained by the MAb were PspA molecules that cross-reacted with

the antiserum to PspC. Besides failing to react with the Mab, it was also noted that PspC bands were of higher molecular weight than the PspA bands. By these criteria the anti-PspC serum cross-reacted with PspA in all strains tested except A66. For A66, a single band was detected. Further testing determined this band to be PspA-derived, rather than PspC-derived. In this case, A66 lacked a *pspC* gene and the PspA of A66 was not reactive with the MAb used, Xi126, even though anti-PspA immune sera does detect PspA in this strain. From the above patterns of reactivity, it was concluded that the PspC polyclonal antiserum is cross-reacting specifically with the PspA molecule.

Example/Result 7: Immunization and challenge studies:

CBA/N mice were immunized with purified recombinant PspC proteins originating from strain L81905 (AA 263-482), the full alpha-helical region of PspC in strain D39 (AA 1-445), or a truncated portion of the PspC protein in strain D39 (AA 255-445). Each mouse received only one of the above recombinant proteins and groups of 5-6 mice were immunized in each experiment. The mice were immunized subcutaneously with approximately 1 µg of purified protein emulsified in 0.2 ml of complete Freund's adjuvant. Three weeks later they were boosted with 1 µg of purified protein in saline. Three weeks after the boost, the mice were challenged with approximately 700 colony-forming units (CFU) of pneumococcal strain WU2. Control mice were immunized with buffer and complete Freund's adjuvant without PspC.

Analysis of Immune Sera: Mice were bled retroorbitally 24 hours before challenge. The blood was collected into .5 ml 1% BSA/phosphate buffered saline. Samples were centrifuged for 1 min (2000 rpm) and the supernatant was collected and stored at -20°C until used in direct ELISAs (enzyme-linked immunosorbent assays). Microtiter 96 well plates (Nunc, Weisbaden, Germany) were coated overnight at 4°C with .5 µg of expressed protein which included PspC (AA 1-445) and PspA (UAB55- AA 1-301, UAB15- AA 1-314 and UAB103- AA 1-370). Plates were blocked with 1% bovine serum albumin/phosphate buffered saline (PBS) followed by incubation with immune sera for 3 hour at 37°C. Plates were washed with PBS/DAKO with .15% tween and incubated with goat anti-mouse immunoglobulin biotin-conjugated antiserum and streptavidin alkaline phosphatase (Southern Biotechnology Assoc., Birmingham AL). They were developed with p-nitrophenyl phosphate (Sigma, St. Louis, MO). The log reciprocal titer giving 33% maximum binding to the mouse immune sera was determined to evaluate the reactivity.

Ability of PspC to elicit protective immunity in mice: Mice were immunized with one of three purified fragments of clade B PspC: L81905 (AA 263-482), D39 (AA 1-445) and D39 (AA 255-445). None of these immunogens contained the *pspA*-like alpha-helical region 2 noted earlier, but all of the immunogens contained the proline-rich region. Mice immunized with PspC and control mice then immunized with adjuvant only were challenged with WU2 or BG7322. WU2 is a capsular serotype 3 strain that produces no detectable PspC and does not contain the structural gene for *pspC* (Figure 6). BG7322 is a capsular serotype 6B strain and contains a clade A PspC molecule. Significant protection against death was seen with both challenge strains in mice immunized with the three different PspC clade B molecules (Table 2). Protective immunity in mice challenged with WU2 must derive from the ability of the PspC immunogen to elicit immunity (presumably mediated by antibodies) in the mice that cross-reacts with the PspA molecule present on surface of strain WU2 because this strain lacks PspC. The ability of PspC to elicit immunity that is directed against PspA was expected from the data herein since PspC had been shown to elicit antibodies cross-reactive with PspA (Figure 6). Protection of the mice challenged with BG7322 was statistically significant even though only 62% of the mice were protected as opposed to 96% when challenged with WU2.

Example/Result 8: Antibody Elicited to Recombinant PspC:

For this study sera was used from mice immunized with LXS240, which encoded amino acids 255-445 of clade B PspC/D39. This sequence contains the entire proline-rich region of PspC/D39. Direct binding ELISAs were conducted to localize the epitope yielding the cross-reactivity with PspA. Microtiter 96 well plates were coated with fragments of PspC/D39 and PspA/Rx1. Each of the cloned PspA/Rx1 molecules used in these assays expressed the PspA alpha-helical region and differed only in the number of the amino acids it contained in the proline-rich region. UAB55 contained 15 amino acids in the proline-rich region, UAB15 contained 26 amino acids in the proline-rich region, and UAB103 contained the entire proline-rich region. The results from the ELISA are depicted in Figure 7. Mouse antisera only reacted with the PspA/Rx1 molecules containing the entire proline-rich region. The antisera did not react with the PspA molecules UAB55 and UAB15 that contained truncated proline regions. These results strongly suggest that the antibodies elicited by PspC that cross-protect against PspA are probably directed at the proline-rich regions of these molecules. Accordingly, the

invention comprehends a method for eliciting anti-PspA antibodies comprising administering PspC or an epitopic region thereof or a polypeptide comprising an epitope of PspC.

Example/Result 9: Modular evolution and chimeric structure of *pspC*:

PspC is a chimeric protein, which has acquired domains from both interspecies and intraspecies genetic exchanges. The protein contains a signal sequence has 75% nucleotide identity to the *bac* gene from group B streptococci (accession numbers X59771 and X58470) (Hammerschmidt et al. 1997). The *bac* gene encodes the *b* antigen of Group B streptococci, a cell surface receptor that binds the constant region of human IgA. This similar sequence in the signal peptide region suggests that potential interspecies genetic exchange between group B streptococci and *S. pneumoniae* may have formed a chimeric locus including the *bac* regulatory region and a partial *pspA* or a *pspA*-like locus to create an ancestral gene for *pspC*. The origin of the central region specific to the current *pspC* genes is unknown. The direct amino acid repeats of the alpha-helix suggest that this region of PspC has evolved by a domain duplication event. This internal duplication of a portion of the alpha-helix led to gene elongation. The region of the alpha-helix is presumably the functional region of the molecule and reportedly binds SIgA (Hammerschmidt et al. 1997). Further intraspecies variation events are hinted at in the finding that 4% of PspC proteins are of clade A. This clade appears to have derived from a recombination event with PspA (or *visa versa*) providing further evidence of chimeric structure of PspC and possibly PspA molecules.

Several functions have been attributed to the PspC molecule. In addition to binding secretory IgA and a moiety on the surface of epithelial cells, Hostetter et al. have reported that PspC binds the complement component C3 (Hostetter et al. 1997). Recent studies have shown that PspA inhibits complement activation by inhibiting the formation of the C3 convertase. With the similar structural domains of PspA and PspC, it is conceivable that the virulence properties of the two proteins may complement each other in the host. WU2 is a strain of *S. pneumoniae* that does not contain a structural gene for PspC. When mutants of PspA are produced in WU2 that lacks PspC there is a 10,000-fold decrease in virulence (Briles et al. 1997). When PspA is mutated in D39, a strain that contains both PspA and PspC, there is only a 10-fold decrease in virulence (Briles et al. 1997). From the data herein, PspA and PspC may complement each other in their abilities to block the clearance of pneumococci by interfering with the complement pathway (see *also* the preliminary data of Hostetter et al. 1997 and the data of Briles et al. 1997).

Rosenow et al. demonstrated that CbpA is expressed more strongly by pneumococci in the nasopharynx than by pneumococci in the blood (Rosenow et al. 1997). Thus, it is feasible that the two molecules may serve the same general function, possibly in different host tissues and in different stages of infection. Furthermore, either molecule may be more critical to virulence in the absence of the other. This hypothesis is further strengthened by data from ongoing studies that show that mutants lacking in both PspC and PspA are significantly decreased in virulence.

In PspC immunization studies, Applicants challenged mice with a strain expressing both PspC and PspA and a strain expressing PspA but not PspC. By including strains lacking the *pspC* gene Applicants could determine if protection elicited by PspC required the expression of PspC or might act, at least in part, through cross-reactions with PspA. For the study presented, mice were immunized with clade B PspC. This molecule lacks the PspA-PspC homology region near the C-terminal end of the alpha-helical region of PspC. Thus, this immunogen was expected to be one that would give less cross-reaction with PspA than would a clade A PspC. Even so, immunization with PspC/D39 resulted in protection when mice were challenged with either strain BG7322 that expresses both PspA and PspC, or with strain WU2, which expresses PspA but lacks PspC.

The protection-eliciting PspC immunogen contained the entire proline-rich region. The alpha-helical regions of PspA/WU2 and PspC/D39 have essentially no homology. However, the proline-rich region of PspC is repetitive and homologous with PspA. It was possible that antibody to this region was responsible for the cross-protection we have observed. This hypothesis was supported by the observation that antibody elicited to PspC reacted with PspA fragments that contained the proline-rich region but not with those that lacked the proline-rich region in direct ELISAs. Antibodies elicited by PspC also cross-reacted with PspA on Western blots. The likelihood that the protective cross-reaction of PspC immune sera is mediated through PspA was further strengthened by the sequence data released by TIGR (accessed by the internet at [http:// www.tigr.org](http://www.tigr.org)). Extensive searches of the largely completed genome failed to find other pneumococcal gene sequences with as high a similarity with the PspC sequence domains as the proline-rich region of PspA.

Electron microscopy surface labeling studies, and epitope mapping studies have localized PspA on the surface of pneumococci with the largely exposed alpha-helical region (Gray, *Pneumococcal infection*, in Bacterial Infection, P.E. Brachman, Ed. 1997, Plenum Pub. Corp.

NY; McDaniel et al. 1994; McDaniel et al, *Monoclonal antibodies against surface components of Streptococcus pneumoniae*, in Monoclonal antibodies against bacteria, A.J.L. Macario and E.C. de Macario, Eds. 1986, Academic Press, Inc. Orlando). Studies by Yother and White have shown that PspA is attached by the C-terminal end to lipoteichoic acids (Yother et al. 1994). No information has been available however, about whether or not the proline-rich domain is surface exposed. Results from these experiments indicating that antibodies to the proline-rich domain are protective suggest that this domain of PspA is probably accessible on the surface of the pneumococci. This study also provides the first published evidence that antibodies reactive with the proline-rich region of PspA can be protective against pneumococcal infection.

PspA, PspC/CbpA/SpsA, LytA and PcpA are proteins of *S. pneumoniae* that contain choline-binding domains. The choline-binding domains of PspC/CbpA/SpsA contain between 4 and 11 repeats of about 20 amino acids. The consensus sequences of these repeats are from 90 to 95% identical. The middle region of the choline-binding domain of PspA and PspC is conserved. The first and last two repeats of PspA and PspC differ substantially (by 40 to 65%) from the consensus sequence. Even so, PspA and PspC sequences in these areas generally have the same deviations from the consensus sequence and in most cases are within 95% identical. The choline-binding domains of LytA and PcpA are quite different from that of PspA or PspC (42-62% identity) (Garcia et al. 1986; Sanchez-Beato et al. 1998). Whereas PspA and PspC have most likely evolved by gene duplication, PcpA has probably arisen from horizontal gene transfer. The choline-binding regions of these proteins all support a modular form of evolution of this group of proteins.

This disclosure provides a comprehensive study of the sequence of *pspC* and shows that PspCs can be divided into two clades based on the sequences in their alpha-helical and proline-rich domains. The disclosure also demonstrates that immunity to the proline-rich domain of PspC can be protective through its recognition of the proline-rich domain of the PspA molecule. The fact that the N-terminal alpha-helical domain of PspC is different from the alpha-helical domain of PspA suggests that PspC and PspA may serve somewhat distinct roles in virulence. However, the fact that the two molecules have a very similar domain structure and have similarity in much of their sequences raises the possibility that these two molecules may have similar functions. Although there are sequences of a few *pspC* alleles, this is the first report that the PspC family contains two clades and that the PspC molecules that contain homology to PspA

within the cross-protective region of the alpha-helix. The identification of two clades of PspC is pertinent to PspC-containing vaccine, immunological or immunogenic compositions, as well as to methods for identifying PspA, *pspA*, PspC, *pspC*, and/or *S. pneumoniae*. Moreover, the observation that antibodies to the proline-rich regions of PspA and PspC can be cross-protective facilitates the design of more efficacious vaccines, as well as of alternate vaccines, immunogenic or immunological compositions, e.g., by providing epitopic regions of PspC, epitopes of PspC and nucleic acid molecules encoding the same, and methods for identifying PspA, *pspA*, PspC, *pspC*, and/or *S. pneumoniae*.

Table 1. Conservation of PspC domains shown as percent amino acid identities.

PspC Domain	Clade A		Clade B	
	PspC vs. PspC	PspC vs. PspA	PspC vs. PspA	PspA vs. PspA
	Orthologous	Paralogous	paralogous	orthologous
Upstream through signal peptide	>97%	No alignment possible	no alignment possible	>95%
Whole gene	67.6-99%	14-29%	14-21%	22-79%
Alpha-helical 1	66.9-99.6%	11.8-22.0%	14.8-23.1%	not present
Alpha-helical 2	100%	13.1-88.7%	not present	14-99%
Proline-rich*	High**	high	high	high
Choline-binding	87%	77%	79.1-99%	77-98%
17 AA tail	100%	88.9%	88.9-94.4%	98-100%
3' downstream	99%	No alignment possible	no alignment possible	N.D.

Percentages calculated using a distance matrix from Paup 3.0.

* All PspA and PspC molecules have a repetitive segment of protein in this region with the motif PEPK or PAPAP. Clade B PspC molecules have a conserved non-repetitive break in the proline-rich region. Distance ranges are uninformative because it is not possible to align these sequences in a meaningful way.

Table 2. Cross-Protection of CBA/N Mice immunized with Recombinant PspC

Immunogen			immunized ²	non-immunized ²	P value ¹
PspC fragment	Capsular Serotype of PspC donor	Challenge strain and Capsular Serotype	# of mice alive/dead ³	# of mice alive/dead ³	
L81905 (AA 263-248)	4	WU2 (3)	13/0	1/12	<.0001
D39 (AA 1-445)	2	WU2 (3)	5/0	0/5	.008
D39 (AA 255-445)	2	WU2 (3)	4/1	0/5	.048
D39 (AA 255-445)	2	BG7322 (6B)	13/8	1/19	.0002

¹ The statistical difference between immunized and non-immunized was calculated using the Fisher exact test.

² Mice were either immunized with PspC with complete Freund's adjuvant or with adjuvant and buffer but no antigen.

³ Mice were challenged 21 days post immunization with 700 CFU of WU2 or 2000 CFU of BG7322 injected i.v. in 0.2 Ringer's injection solution.

* * *

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

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WHAT IS CLAIMED IS:

1. An isolated and/or purified polypeptide comprising at least one epitope or epitopic region of PspC.
2. The polypeptide of claim 1 which is shorter than natural or full length PspC or PspA.
3. The polypeptide of claim 1 selected from the group consisting of: the alpha helical region, the proline region, the combination of the alpha helical and proline regions, the entire PspC molecule, amino acid(s) ("aa") of PspC clade A 1-590, 1-204, 46-204, 1-295, 46-295, 1-454, 46-454, 204-454, 295-454, 1-590, 46-590, 204-590, 295-590, 454-590, 1-652, 46-652, 204-652, 295-652, 454-652, 590-652, 1-892, 46-892, 204-892, 295-892, 454-892, 590-892, aa of PspC clade B 1-664, 1-375, 1-445, 1-101, 1-193, 1-262, 1-355, 101-193, 101-262, 101-355, 101-375, 101-455, 193-262, 193-355, 193-375, 193-445, 262-355, 262-375, 262-445, 355-375, 355-445, 375-445, 101-664, 193-664, 262-664, 355-664, 375-664, 1-end of proline subregion A, 1-beginning of proline subregion B, 101-end of proline subregion A, 101-beginning of proline subregion B, 193-end of proline subregion A, 193-beginning of proline subregion B, 262-end of proline subregion A, 262-beginning of proline subregion B, 355-end of proline subregion A, 355-beginning of proline subregion B, 375-end of proline subregion A, or proline subregion A, 375-beginning of proline subregion B, proline subregion B, beginning of proline subregion B-aa 664, 263-482, 1-445 and 255-445.
4. An immunogenic, immunological or vaccine composition comprising a polypeptide as claimed in any one of claims 1-3.
5. The composition of claim 4, further comprising at least one additional pneumococcal antigen or epitope of interest.
6. The composition of claim 5 wherein the at least one additional pneumococcal antigen or epitope is at least one different PspC or fragment thereof containing at least one epitope of PspC.
7. The composition of claim 5 wherein the at least one additional pneumococcal antigen or epitope is at least one PspA or fragment thereof containing at least one epitope of PspC.
8. The composition of claim 5 comprising the polypeptide being from PspC clade A, at least one different PspC or fragment thereof containing at least one epitope of PspC from PspC

clade B, and at least two different PspAs or fragments thereof containing at least one epitope of PspA.

9. The composition of claim 4 further including an adjuvant.
10. An isolated and/or purified nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide as claimed in any one of claims 1-3.
11. The nucleic acid molecule of claim 10 which is DNA.
12. A vector or plasmid comprising the isolated nucleic acid molecule of claim 10.
13. A vaccine, immunological or immunogenic composition comprising the vector or plasmid of claim 12.
14. A method for eliciting an immunological response against *Streptococcus pneumoniae* comprising administering a polypeptide as claimed in any one of claims 1-3.
15. A method for eliciting an immunological response against *Streptococcus pneumoniae* comprising administering a composition as claimed in claim 4.
16. A method for eliciting an anti-PspA antibody comprising administering a polypeptide as claimed in any one of claims 1-3.
17. A method for eliciting an anti-PspA antibody comprising administering a composition as claimed in claim 4.
18. A method for eliciting an immunological response against *Streptococcus pneumoniae* comprising administering a composition as claimed in claim 13.
19. A method for eliciting an anti-PspA antibody comprising administering a composition as claimed in claim 13.
20. The method of claim 14 performed by administering an injection, or by oral, nasal, or mucosal administration.
21. The method of claim 15 performed by administering an injection, or by oral, nasal, or mucosal administration.
22. The method of claim 16 performed by administering an injection, or by oral, nasal, or mucosal administration.
23. The method of claim 17 performed by administering an injection, or by oral, nasal, or mucosal administration.
24. The isolated nucleic acid molecule of claim 10 which is a probe or primer for detecting *pspC*, or *pspA*, or both *pspC* and *pspA*, or *Streptococcus pneumoniae*.

25. A method for detecting *pspC*, or *pspA*, or both *pspC* and *pspA*, or *Streptococcus pneumoniae* comprising contacting the isolated nucleic acid molecule of claim 24 with a sample and detecting hybridization, whereby hybridization is indicative of the presence of *pspC*, or *pspA*, or both *pspC* and *pspA*, or *Streptococcus pneumoniae*.

26. A method for preparing a PspC protein or fragment thereof comprising obtaining expression thereof from the vector or plasmid of claim 12.

27. A method for preparing an immunogenic, immunological or vaccine composition comprising admixing a polypeptide as claimed in any one of claims 1-3 with a carrier or diluent and optionally an adjuvant.

ABSTRACT OF THE DISCLOSURE

Disclosed and claimed are: epitopic regions of Pneumococcal Surface Protein C or "PspC", different clades of PspC, isolated and/or purified nucleic acid molecules such as DNA encoding a fragment or portion of PspC such as an epitopic region of PspC or at least one epitope of PspC, uses for such nucleic acid molecules, e.g., to detect the presence of PspC or of *S. pneumoniae* by detecting a nucleic acid molecule therefor in a sample such as by amplification and/or a polymerase chain reaction, vectors or plasmids which contain and/or express such nucleic acid molecules, e.g., *in vitro* or *in vivo*, immunological, immunogenic or vaccine compositions including at least one PspC and/or a portion thereof (such as at least one epitopic region of at least one PspC and/or at least one polypeptide encoding at least one epitope of at least one PspC), either alone or in further combination with at least one second pneumococcal antigen, such as at least one different PspC and/or a fragment thereof and/or at least one PspA and/or at least one epitopic region of at least one PspA and/or at least one polypeptide including at least one epitope of PspA. PspC or a fragment thereof, and thus a composition including PspC or a fragment thereof, can be administered by the same routes, and in approximately the same amounts, as PspA. Thus, the invention further provides methods for administering PspC or a fragment thereof, as well as uses of PspC or a fragment thereof to formulate such compositions.

45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

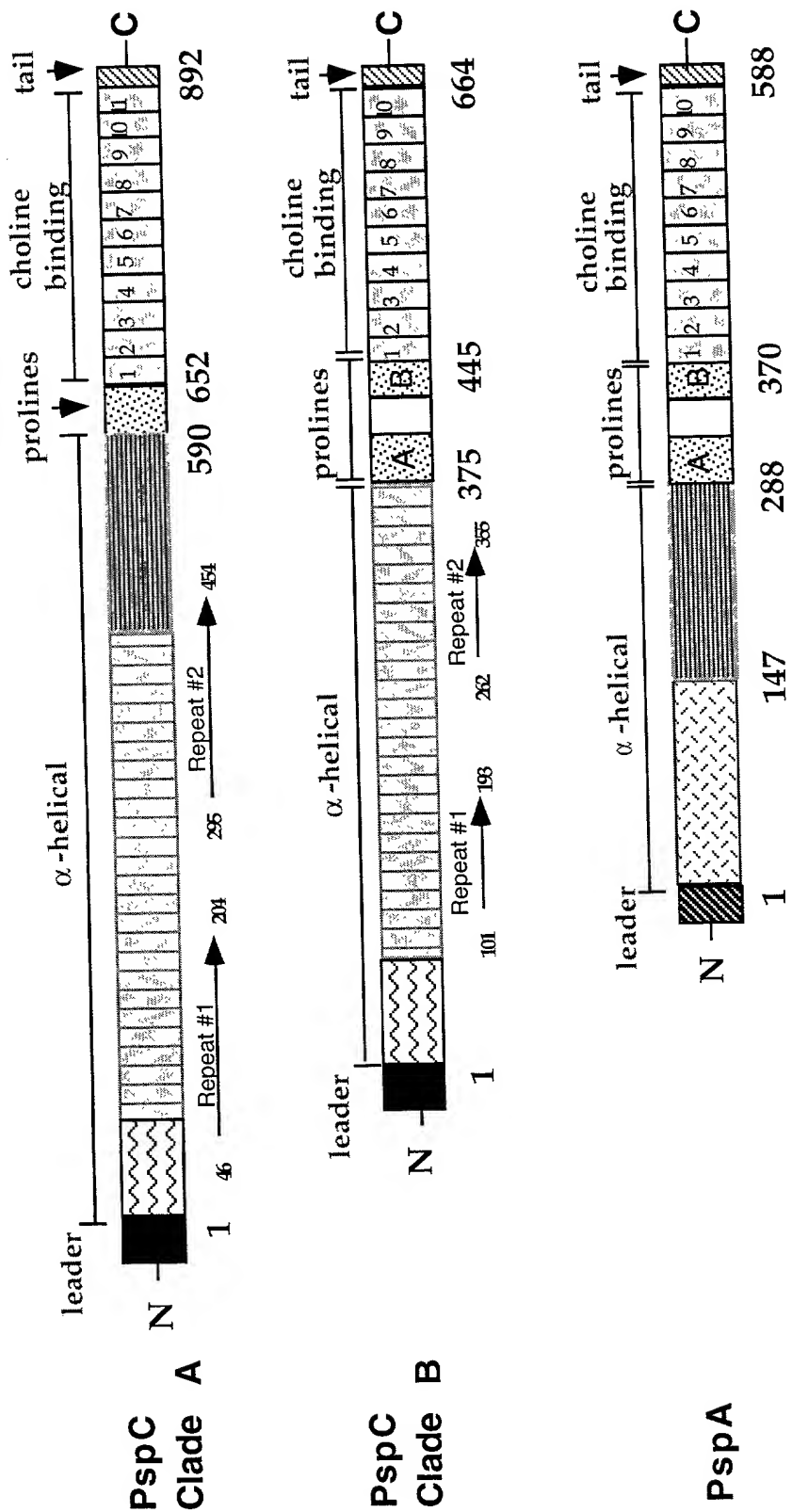
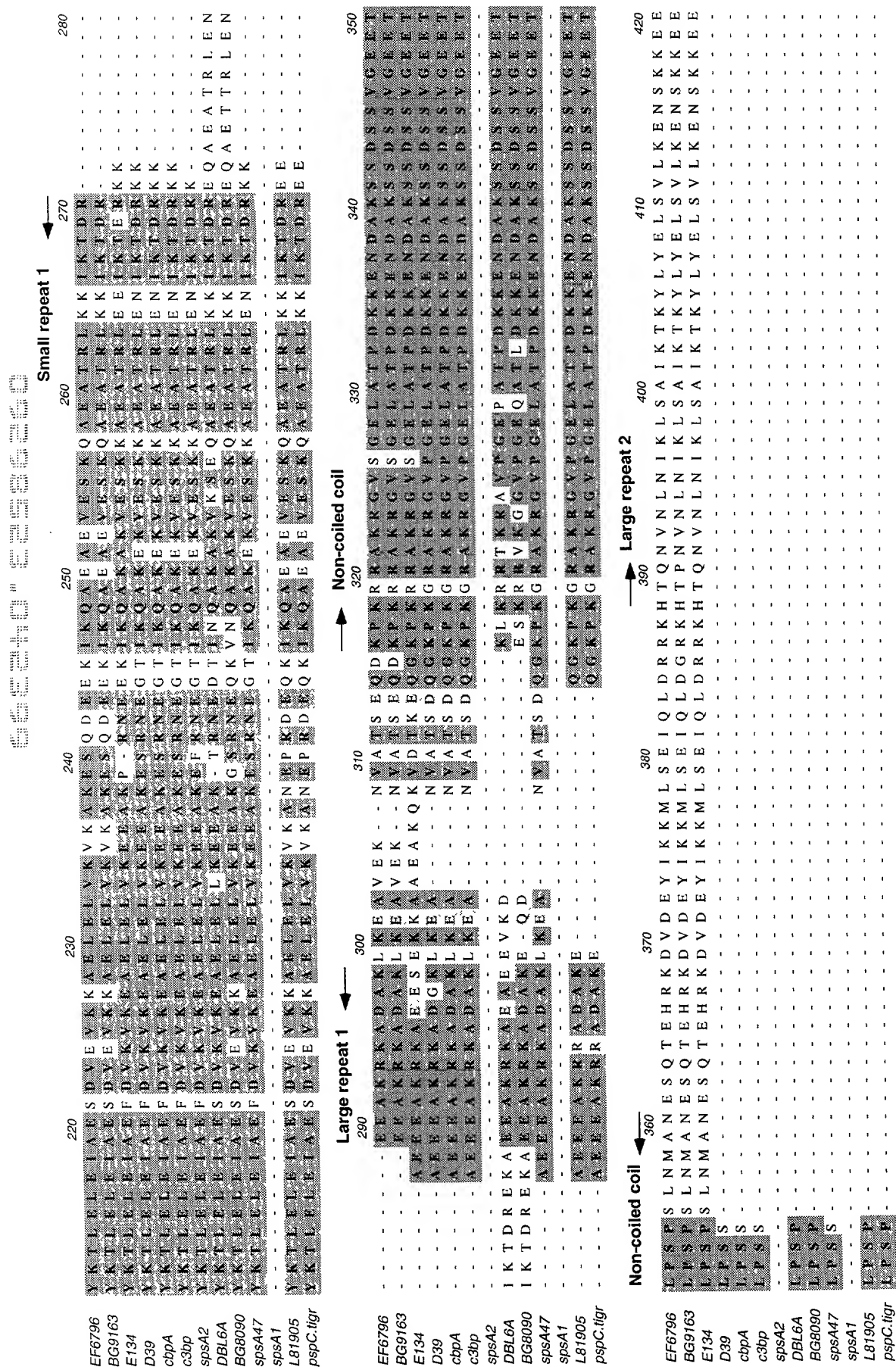


Figure 1: Cartoon of the PspC Clades compared to a representative PspA molecule. Long arrows represent the direct repeats found within α helix. The hypervariable region is indicated by the zig-zag lines. The region of homology found with the alpha helix is indicated by the horizontal lines.

Fig. 1



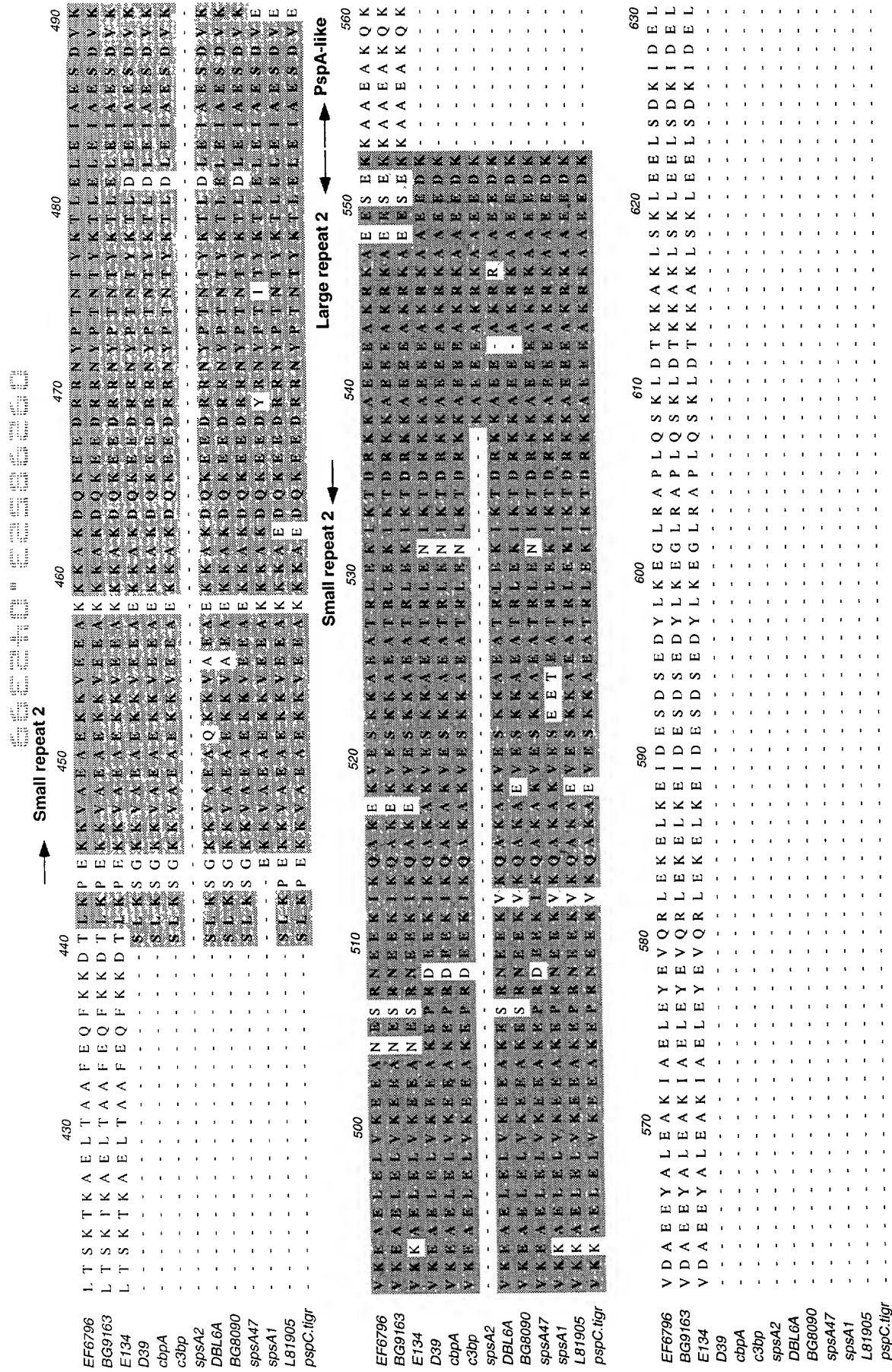


Fig. 2 (cont'd)

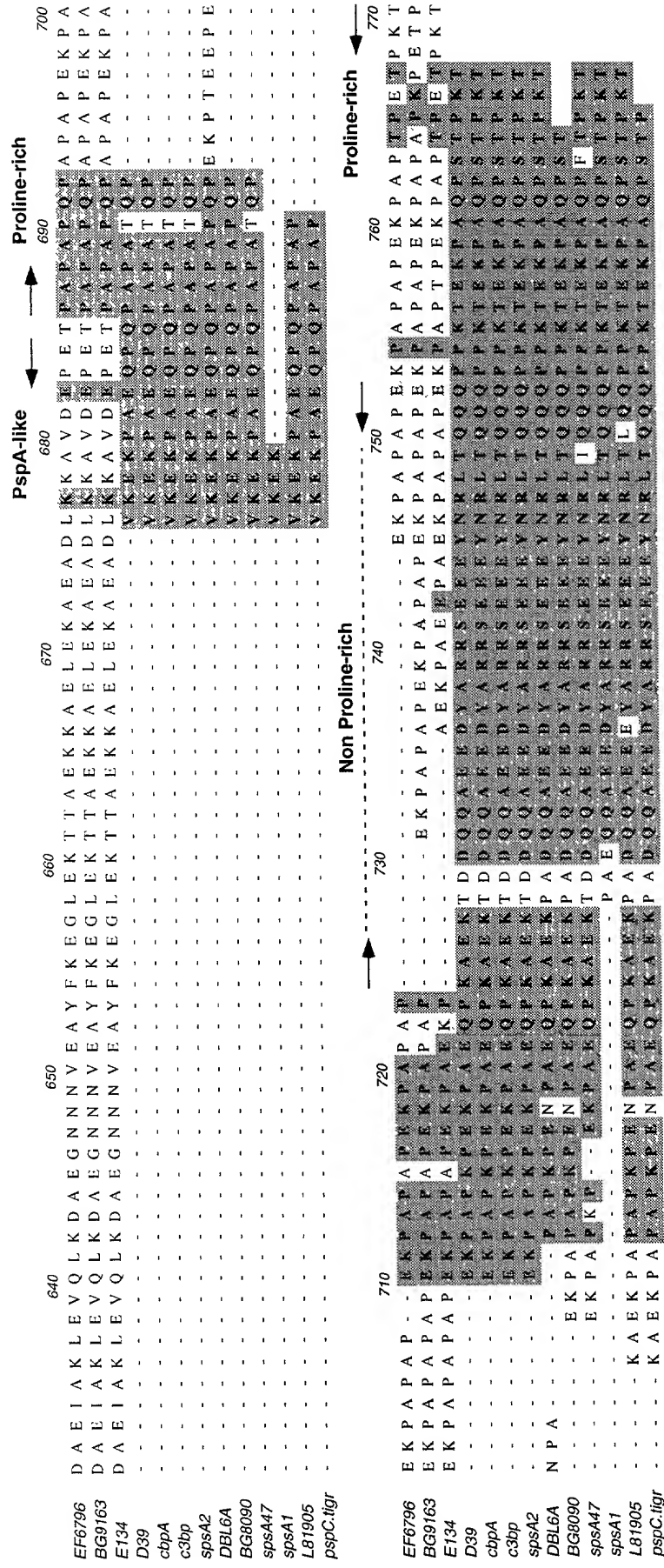


Fig. 2 (cont'd)

	a	b	c	d	e	f	g	
1								Glu Gly Val Arg Ser Gly Asn Asn Leu Thr
11								Val Thr Ser Ser Gly
16		Gln	Asp	Ile	Ser	Lys	Lys	
22	Tyr	Ala	Asp	Glu	Val	Glu	Ser	
29			His	Leu	Glu	Ser	Ile	
34	Leu	Lys	Asp	Val	Lys	Lys	Asn	
41	Leu	Lys	Lys					
44	Val	Gln	His	Thr	Gln	Asn	Val	
51			Gly	Leu	Ile	Thr	Lys	
56	Leu	Ser	Glu	Ile	Lys	Lys	Lys	
63							Tyr	
64	Leu	Tyr	Asp	Leu	Lys			
69	Val	Asn	Val	Leu	Ser	Glu	Ala	
76			Glu	Leu	Thr	Ser	Lys	
81				Thr	Lys	Glu	Thr	
85	Lys	Glu	Lys	Leu	Thr	Ala	Thr	
92	Phe	Glu	Gln	Phe	Lys	Lys	Asp	
99								Thr Leu Pro Thr Glu Pro
105					Glu	Lys	Lys	
108	Val	Ala	Glu	Ala	Gln	Lys	Lys	
115	Val	Glu	Glu	Ala	Lys	Lys	Lys	
122				Ala	Glu	Asp	Gln	
126	Lys	Glu	Lys	Asp	Arg	Arg	Asn	
133	Tyr	Pro	Thr	Ile	Thr			
138	Tyr	Lys	Thr	Leu	Glu	Leu	Glu	
145	Ile	Ala	Glu	Ser	Asp	Val	Glu	
152	Val	Lys	Lys	Ala	Glu	Leu	Glu	
159	Leu	Val	Lys	Val	Lys	Ala	Lys	
166	Glu	Ser	Gln	Asp	Glu	Glu	Lys	
173	Ile	Lys	Gln	Ala	Glu	Ala	Glu	
180	Val	Glu	Ser	Lys	Gln	Ala	Glu	
187				Ala	Thr	Arg		
190	Leu	Lys	Lys	Ile	Lys	Thr	Asp	
197	Arg	Glu	Glu	Ala	Lys	Arg	Lys	
204		Ala	Asp	Ala	Lys	Leu	Lys	
210		Glu	Ala	Val	Glu	Lys	Asn	
216	Val	Ala	Thr	Ser	Glu	Gln	Asp	
223	Lys							Pro Lys Arg Arg Ala Lys Arg Gly Val Ser
224								Gly Glu Leu Ala Thr Pro Asp Lys Lys Glu
234								Asn Asp Ala Lys Ser Ser Asp Ser Ser Val
244								Gly Glu Glu Thr Leu Pro Ser Pro Ser Leu
254								Asn Met Ala Asn
264								
268					Glu	Ser	Gln	
271		Thr	Glu	His	Arg	Lys	Asp	
277	Val	Asp	Glu	Tyr				
281	Ile	Lys	Lys	Met	Leu	Ser	Glu	
288	Ile	Gln	Leu	Asp	Arg	Arg	Lys	
295			His	Thr	Gln	Asn	Val	
300			Asn	Leu	Asn	Ile	Lys	
305	Leu	Ser	Ala	Ile	Lys	Thr	Lys	
312				Tyr	Leu	Tyr	Glu	
316	Leu	Ser	Val	Leu	Lys	Glu	Asn	
323					Ser	Lys		
325	Lys	Glu	Glu	Leu	Thr	Ser	Lys	
332				Thr	Lys	Ala	Glu	
336	Leu	Thr	Ala	Ala	Phe	Glu	Gln	

Fig. 3

page 1

343	Phe Lys Lys	
346		Asp Thr Leu Lys Pro
351		Glu Lys Lys
354	Val Ala Glu Ala Glu Lys Lys	
361	Val Glu Glu Ala Lys Lys Lys	
368		Ala Lys Asp Gln
372	Lys Glu Glu Asp Arg Arg Asn	
379		Tyr
380		Pro Thr Asn Thr
384	Tyr Lys Thr Leu Glu Leu Glu	
391	Ile Ala Glu Ser Asp Val Lys	
398	Val Lys Glu Ala Glu	
403	Leu Glu Leu Val Lys Glu Glu	
410	Ala Asn Glu Ser Arg Asn Glu	
417		Glu Lys Ile Lys Gln Ala
423	Lys Glu Lys Val Glu Ser Lys	
430	Lys Ala Glu Ala Thr Arg	
436	Leu Glu Lys Ile Lys Thr Asp	
443	Arg Lys Lys Ala Glu Glu Glu	
450		Ala Lys Arg Lys
454	Ala Glu Glu Ser Glu Lys Lys	
461	Ala Ala Glu Ala Lys Gln Lys	
468	Val Asp Ala Glu Glu Tyr Ala	
475		Leu Glu Ala Lys
479	Ile Ala Glu Leu Glu Tyr Glu	
486	Val Gln Arg Leu Glu Lys Glu	
493	Leu Lys Glu	
496	Ile Asp Glu Ser Asp Ser Glu	
503		Asp Tyr Leu Lys Glu Gly
509	Leu Arg Ala	
512		Pro Leu Gln Ser Lys
517	Leu Asp Thr Lys Lys Ala Lys	
524	Leu Ser Lys	
527	Leu Glu Glu Leu Ser Asp Lys	
534	Ile Asp Glu Leu Asp Ala Glu	
541	Ile Ala Lys Leu Glu Val Gln	
548	Leu Lys Asp Ala Glu Gly Asn	
555		Asn Asn
557	Val Glu Ala Tyr Phe Lys Glu	
564		Gly Leu Glu Lys Thr
569		Thr Ala Glu Lys Lys
574		Ala Glu Leu Glu Lys Ala
580	Glu Ala Asp Leu Lys Lys Ala	
587	Val Asp Glu	

Figure 3. The coiled-coil motif of the α -helix of PspC. Amino acids that are not in the coiled-coil motif are in the right column. This is the output from the Matcher program accessed through the Internet (<http://catt.poly.edu/~jps/>).

Fig. 3 (cont'd)

Clade B

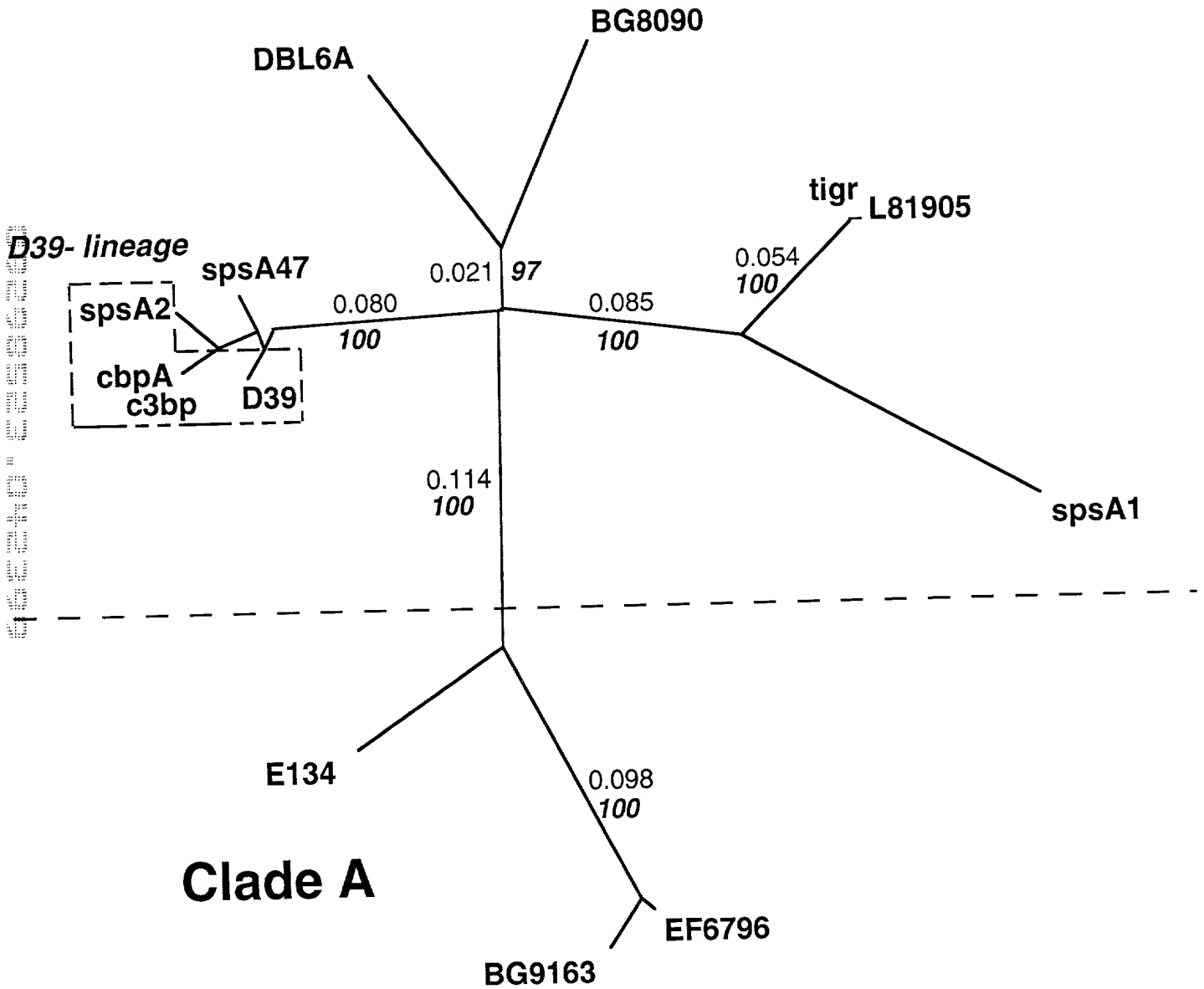


Fig. 4

— 0.05 changes

FIGURE 5. Choline-binding Domain

PspC consensus

111111111112
12345678901234567890
1 KTGWKQENGWYFYNTDGSMA
2 TGWLQNNGSWYYLNAMGAMA
3 TGWLQNNGSWYYLNANGSMA
4 TGWLQNNGSWYYLNANGAMA
5 TGWLQYNGSWYYLNANGDMA
6 TGWLQYNGSWYYLNSNGAMA
7 TGWLQYNGSWYYLNANGDMA
8 TGWLQNNGSWYYLNANGDMA
9 TGWLQYN SWYYLNANGDMA
10 TGWVKDGDWYYLEASGAMKA
11 SQWFKVSDKWYYVNGSGALA
VNTTVDGYGVNANGGEWVN 18 amino acid tail PspC
28% identical to consensus

TGWLQNNGSWYYLNANGAMA PspC consensus repeat
Y

For PspA/R36A alignment see Yother and Briles 1992.

TGWLQXNGSWYYLNANGAMA PspA/Rx1 consensus
Y 95% identical to PspC consensus

VNTTVDGYKVNANGGEWV_ PspA/Rx1 17 amino acid tail.
One AA different from PspC,
And one AA shorter.

Fig. 5

PspA/EF5668

11111111112
12345678901234567890
1 IGWKQENGMWYFYNTDGSM A
2 TGWLQNNGSWYYLNSNGAM A
3 TGWLQYNGSWYYLNANGAM A
4 TGWLQYNGSWYYLNANGAM A
5 TGWLQYNGSWYYLNANGDM A
6 TGWLQYNGSWYYLNANGDM A
7 TGWAKVHGSWYYLNANGSM A
8 TGWVKDGETWYYLEASGSMKA
9 NQWFQVSDKWYYVNGLGSL S
10 VNTTVDGYKVNANGEWV 17 amino acid tail EF5668 PspA

TGWLQYNGSWYYLNANGSM A PspA/EF5668 consensus
90% identical to PspC consensus

Repeat #1

PspC consensus	KTGWKQENGNWYFYNTDGSM A
PspA/Rx1	TGWKQENGMWYFYNTDGSM A
PspA/EF5668	IGWKQENGMWYFYNTDGSM A

Repeat #N-1

PspC consensus	TGWVKDGDWYYLEASGAMKA
PspA/Rx1	TGWVKDGDWYYLEASGAMKA
PspA/EF5668	TGWVKDGETWYYLEASGSMKA

Repeat #N

PspC consensus	SQWFKVSDKWYYVNGSGALA
PspA/Rx1	SQWFKVSDKWYYVNGLGALA
PspA/EF5668	NQWFQVSDKWYYVNGLGSL S

17-18 AA tail

PspC consensus	VNTTVDGYGVNANGEWVN
PspA/Rx1	VNTTVDGYKVNANGEWV
PspA/EF5668	VNTTVDGYKVNANGEWV

Fig. 5 (cont'd)

Log Reciprocal titer of serum from PspC-immunized mice

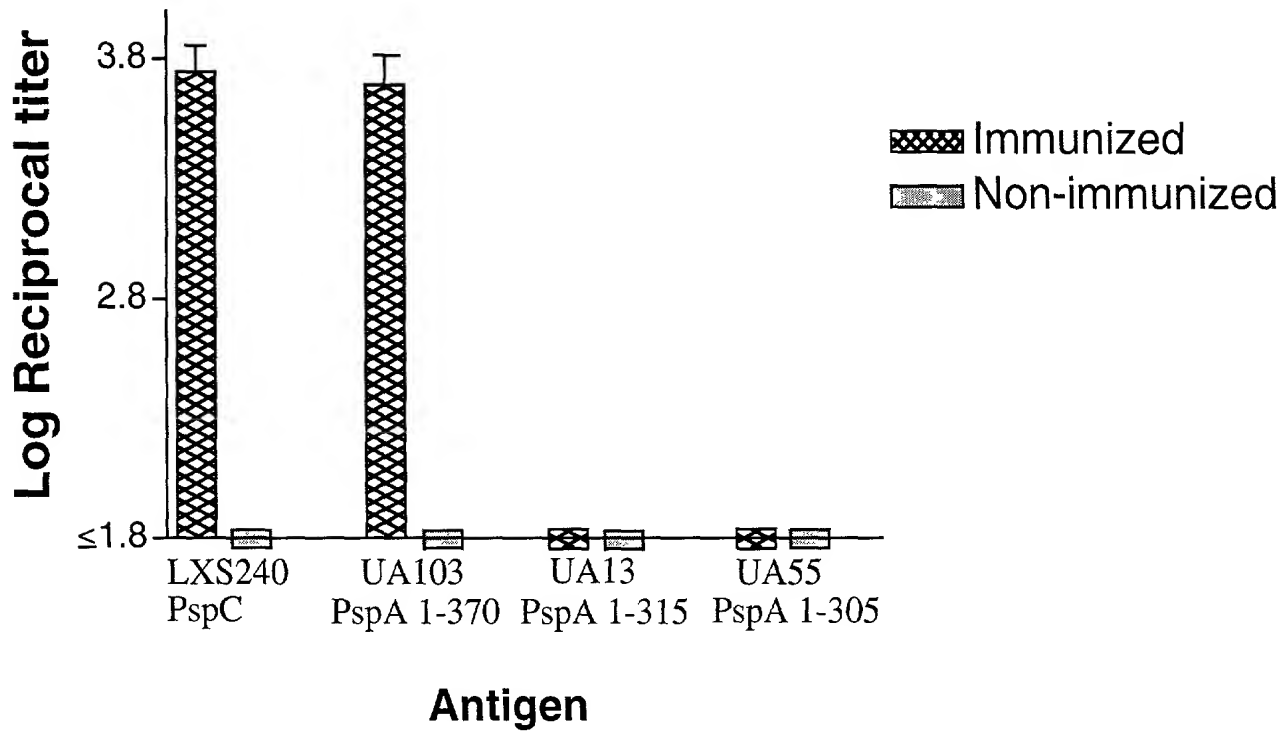


Figure 7. Level of antibody reactive to PspC and PspA fragments present in the sera of mice immunized with PspC. Each bar represents the mean of the log reciprocal titer and upperbound of standard error of sera from five mice. The limit of detection of the log reciprocal antibody titer is 1.8.

FIG. 7

Fig. 8

Other Formats:

Links:

LOCUS CAA05158 539 aa BCT 14-OCT-1997

DEFINITION SpsA protein.

ACCESSION CAA05158

PID g2576331

VERSION CAA05158.1 GI:2576331

DBSOURCE embl locus SPSPSA2, accession AJ002054.1

KEYWORDS

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Bacteria; Firmicutes; Bacillus/Clostridium group; Streptococcaceae;
Streptococcus.

REFERENCE 1 (residues 1 to 539)

AUTHORS Hammerschmidt,S.

TITLE Direct Submission

JOURNAL Submitted (13-OCT-1997) Hammerschmidt S., Microbiology, Division
Microbial Pathogenesis, National Research Centre for Biotechnology,
Spielmannstrasse 7, 38106 Braunschweig, GERMANY

REFERENCE 2 (residues 1 to 539)

AUTHORS Hammerschmidt,S., Talay,S.R., Brandtzaeg,P. and Chhatwal,G.S.

TITLE SpsA, a novel pneumococcal surface protein with specific binding to
secretory immunoglobulin A and secretory component

JOURNAL Mol. Microbiol. 25 (6), 1113-1124 (1997)

MEDLINE 98010350

FEATURES Location/Qualifiers

source 1..539

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/product="SpsA protein"

CDS 1..539

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121 akldaafekf kkdtkpgek vaeakkkvee akkkaedqke edrrnyptnt ykteleiaie
181 fdvkvkeael elvkeeakef nregtikqak ekveskkaea trleniktdr kkaeeekrk
241 aaeedkvkek paeqpqpapa tqpekpapkp ekpaeqpkae ktddqqaed yarrseeeyn
301 rltqqpppkt ekpaqpstpk tgwkqengmw yfyntdgsma tgwlqnngsw yylnangama
361 tgwlqnngsw yylnangama tgwlqnngsw yylnangama tgwlqyngsw yylnsngama

421 tglwlyngsw yylngangma tglwlyngsw yylngangma tglwlyngsw yylngangma
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Other Formats:

Links:

LOCUS SPSPSA2 1620 bp DNA BCT 14-OCT-1997

DEFINITION Streptococcus pneumoniae SIgA binding.

ACCESSION AJ002054

NID g2576330

VERSION AJ002054.1 GI:2576330

KEYWORDS SpsA protein.

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Bacteria; Firmicutes; Bacillus/Clostridium group; Streptococcaceae;
Streptococcus.

REFERENCE 1 (bases 1 to 1620)

AUTHORS Hammerschmidt,S.

TITLE Direct Submission

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Spielmannstrasse 7, 38106 Braunschweig, GERMANY

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secretory immunoglobulin A and secretory component

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MEDLINE 98010350

FEATURES Location/Qualifiers

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DQKE

EDRRNYPTNTYKTLELEIAEFDVKVKEAELELVKEEAKFRNEGTIKQAKEKVES
KKA

EATRLENIKTDRKKAEEEEAKRKAAEEDKVKEKPAEQQPAPATQPEKPAPKPEK
PAEQ

PKAEKTDDQQAEEEDYARRSEEEYNRLTQQQPPKTEKPAQPSTPKTGWKQENGM
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GWLQNNGSW

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/rpt_type=DIRECT

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121 gaggaagta cccaagcagc cacttcttct aatatggcaa agacagaaca taggaaagct
181 gctaaacaag tcgtcgatga atatatagaa aaaatgttga gggagattca actagataga
241 agaaaacata cccaaatgt cgccttaaac ataaagtga gcgcaattaa aacgaagtat
301 ttgcgtgaat taaatgttt agaagagaag tcgaaagatg agttgccgtc agaaataaaa
361 gcaaagttag acgcagcttt tgagaagttt aaaaagata cattgaaacc aggagaaaaa
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481 gaagatcgtc gtaactacc aaccaatact tacaaaacgc ttgaactga aattgctgag
541 ttcgatgtga aagttaaaga agcggagctt gaactagtaa aagaggaagc taaagaattt
601 cgaaacgagg gcacaattaa gcaagcaaaa gagaaagtg agagtaaaaa agctgaggct
661 acaaggtagt aaaacatcaa gacagatcgt aaaaagcag aagaagaagc taaacgaaaa
721 gcagcagaag aagataaagt taaagaaaaa ccagctgaac aaccacaacc agcgccggct
781 actcaaccag aaaaaccagc tccaaaacca gagaagccag ctgaacaacc aaaagcagaa
841 aaacagatg atcaacaagc tgaagaagac tatgtctgta gatcagaaga agaataaat
901 cgttgactc aacagcaacc gccaaaact gaaaaaccag cacaaccatc tactccaaaa
961 acaggctgga aacaagaaaa cggatatgtg tacttctaca atactgatgg tcaatggca
1021 acaggatggc tccaaaacaa cggttcatgg tactatctaa acgctaattg tgctatggcg
1081 acaggatggc tccaaaacaa tggttcatgg tactatctaa acgctaattg tcaatggca
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Fig. 8 (cont'd)

page 5

1201 acaggatggc tccaatacaa tgggtcatgg tactacctaa acagcaatgg cgctatggcg
1261 acaggatggc tccaatacaa tgggtcatgg tactacctca acgctaattg tgatatggcg
1321 acaggatggc tccaaaacaa cgggtcatgg tactacctca acgctaattg tgatatggcg
1381 acaggatggc tccaatacaa cgggtcatgg tattacctca acgctaattg tgatatggcg
1441 acaggatggc tgaagatgg agatacctgg tactatcttg aaacatcagg tgctatgaaa
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Fig. 9

Other Formats:

Links:

LOCUS CAA05159 581 aa BCT 14-OCT-1997

DEFINITION SpsA protein.

ACCESSION CAA05159

PID g2576333

VERSION CAA05159.1 GI:2576333

DBSOURCE embl locus SPSPSA47, accession AJ002055.1

KEYWORDS .

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Bacteria; Firmicutes; Bacillus/Clostridium group; Streptococcaceae;
Streptococcus.

REFERENCE 1 (residues 1 to 581)

AUTHORS Hammerschmidt,S.

TITLE Direct Submission

JOURNAL Submitted (13-OCT-1997) Hammerschmidt S., Microbiology, Division
Microbial Pathogenesis, National Research Centre for Biotechnology,
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MEDLINE 98010350

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 /strain="type 47"
 /db_xref="taxon:1313"

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CDS 1..581
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 121 akldaafekf kkdtlkpgek vaeakkkvee akkkaedqke edrrnyptnt yktleleiae
 181 fdvkvkeael elvkeeakes mregtikqak ekveskkaea trleniktdr kkaeeekrk
 241 adaklkeanv atsdqgkpkg rakrgvpgel atpdkkenda kssdssvgee tlpssslksg
 301 kkvaeakkv eeaekkadq keedrrnypt ntyktldlei aedsvkvkea elelvkeeak
 361 eprdeekikq akakveskka eatrlenikt drkkaeeek rkaaeedkvk ekpaeqpqpa

421 patqpekpap kpekpaepk aektdqqaed yarrsee ynrlqqppp ktekpaqpf
481 pktgwqeng mwyfyntdgs matgwlyng swyynangd matgwkdgd twyyleasga
541 mkasqwfkvs dkwyvngsg alavnttdg ygvnangewv n

//

Fig. 9 (cont'd)

the above report in format

421 patqpekpap kpekpaepk aektdqqaed yarrsee ynrlqqppp ktekpaqpf
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Fig. 9 (cont'd)

page 3

Other Formats:

Links:

LOCUS SPSPSA47 1746 bp DNA BCT 14-OCT-1997

DEFINITION Streptococcus pneumoniae SIgA binding.

ACCESSION AJ002055

NID g2576332

VERSION AJ002055.1 GI:2576332

KEYWORDS SpsA protein.

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Bacteria; Firmicutes; Bacillus/Clostridium group; Streptococcaceae;
Streptococcus.

REFERENCE 1 (bases 1 to 1746)

AUTHORS Hammerschmidt,S.

TITLE Direct Submission

JOURNAL Submitted (13-OCT-1997) Hammerschmidt S., Microbiology, Devision
Microbial Pathogenesis, National Research Centre for Biotechnology,
Spielmannstrasse 7, 38106 Braunschweig, GERMANY

REFERENCE 2 (bases 1 to 1746)

AUTHORS Hammerschmidt,S., Talay,S.R., Brandtzaeg,P. and Chhatwal,G.S.

TITLE SpsA, a novel pneumococcal surface protein with specific binding to
secretory immunoglobulin A and secretory component

JOURNAL Mol. Microbiol. 25 (6), 1113-1124 (1997)

MEDLINE 98010350

FEATURES Location/Qualifiers

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CDS 1..1746
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BASE COUNT 722 a 290 c 408 g 326 t

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181 gctaaacaag tcgtcgatga atatatagaa aaaatgttga gggagattca actagataga
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Fig. 9 (cont'd)

the above report in format

Fig. 10

ther Formats:

Links:

LOCUS AAB70838 663 aa BCT 16-SEP-1997

DEFINITION choline binding protein A.

ACCESSION AAB70838

PID g2425109

VERSION AAB70838.1 GI:2425109

DBSOURCE locus AF019904 accession AF019904.1

KEYWORDS .

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Eubacteria; Firmicutes; Low G+C gram-positive bacteria;

Streptococcaceae; Streptococcus.

REFERENCE 1 (residues 1 to 663)

AUTHORS Rosenow,C., Ryan,P., Weiser,J.N., Johnson,S., Fontan,P.,
Ortqvist,A. and Masure,H.R.

TITLE Contribution of novel choline-binding proteins to adherence,
colonization and immunogenicity of Streptococcus pneumoniae

JOURNAL Mol. Microbiol. (1997) In press

REFERENCE 2 (residues 1 to 663)

AUTHORS Rosenow,C., Ryan,P., Weiser,J.N., Johnson,S., Fontan,P.,
Ortqvist,A. and Masure,H.R.

TITLE Direct Submission

JOURNAL Submitted (18-AUG-1997) Infectious Diseases, St. Jude Children's
Research Hospital, 322 North Lauderdale, Memphis, TN 38105, USA

COMMENT Method: conceptual translation supplied by author.

FEATURES Location/Qualifiers

source 1..663
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/name="CbpA"
CDS 1..663
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/transl_table=11

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181 eatrlenikt drkkaeeak rkadakilkea nvatsdggkp kgrakrgvpg elatpdkken
241 dakssdsvg eetlpsslk sgkkvaeaek kveeaekkak dqkeedrrny ptntyktldl
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the above report in format

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Other Formats:

Links:

LOCUS AF019904 2480 bp DNA BCT 22-SEP-1997

DEFINITION Streptococcus pneumoniae choline binding protein A (cbpA) gene,
partial cds.

ACCESSION AF019904

NID g2425108

VERSION AF019904.1 GI:2425108

KEYWORDS .

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Eubacteria; Firmicutes; Low G+C gram-positive bacteria;

Streptococcaceae; Streptococcus.

REFERENCE 1 (bases 1 to 2480)

AUTHORS Rosenow,C., Ryan,P., Weiser,J.N., Johnson,S., Fontan,P.,
Ortqvist,A. and Masure,H.R.

TITLE Contribution of novel choline-binding proteins to adherence,
colonization and immunogenicity of Streptococcus pneumoniae

JOURNAL Mol. Microbiol. (1997) In press

REFERENCE 2 (bases 1 to 2480)

AUTHORS Rosenow,C., Ryan,P., Weiser,J.N., Johnson,S., Fontan,P.,
Ortqvist,A. and Masure,H.R.

TITLE Direct Submission

JOURNAL Submitted (18-AUG-1997) Infectious Diseases, St. Jude Children's
Research Hospital, 322 North Lauderdale, Memphis, TN 38105, USA

FEATURES Location/Qualifiers

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BASE COUNT 933 a 452 c 575 g 520 t

ORIGIN

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the above report in format

Fig. 11

Other Formats:

Links:

LOCUS AAD00184 929 aa BCT 07-OCT-1996

DEFINITION surface protein C.

ACCESSION AAD00184

PID g4097980

VERSION AAD00184.1 GI:4097980

DBSOURCE locus SPU72655 accession U72655.1

KEYWORDS .

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Eubacteria; Firmicutes; Low G+C gram-positive bacteria;

Streptococcaceae; Streptococcus.

REFERENCE 1 (residues 1 to 929)

AUTHORS Brooks-Walter,A., Tart,R.C., Briles,D.E. and Hollingshead,S.K.

TITLE The pspC gene encodes a second pneumococcal surface protein
homologous to the protection-eliciting PspA protein of
Streptococcus pneumoniae

JOURNAL Unpublished

REFERENCE 2 (residues 1 to 929)

AUTHORS Brooks-Walter,A., Tart,R.C., Briles,D.E. and Hollingshead,S.K.

TITLE Direct Submission

JOURNAL Submitted (26-SEP-1996) Microbiology, University of Alabama at
Birmingham, 19th Street South, Box 10, Birmingham, AL 35294-2170,
USA

FEATURES Location/Qualifiers

source 1..929
 /organism="Streptococcus pneumoniae"
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Protein 1..929
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CDS 1..929
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the above report in format

Fig. 1B (cont'd)

Fig. 11 (cont'd)

Other Formats:

Links:

LOCUS SPU72655 3463 bp DNA BCT 02-JAN-1999

DEFINITION Streptococcus pneumoniae surface protein C (pspC) gene, complete cds.

ACCESSION U72655

NID g4097979

VERSION U72655.1 GI:4097979

KEYWORDS .

SOURCE Streptococcus pneumoniae.

ORGANISM Streptococcus pneumoniae

Eubacteria; Firmicutes; Low G+C gram-positive bacteria;

Streptococcaceae; Streptococcus.

REFERENCE 1 (bases 1 to 3463)

AUTHORS Brooks-Walter,A., Tart,R.C., Briles,D.E. and Hollingshead,S.K.

TITLE The pspC gene encodes a second pneumococcal surface protein homologous to the protection-eliciting PspA protein of Streptococcus pneumoniae

JOURNAL Unpublished

REFERENCE 2 (bases 1 to 3463)

AUTHORS Brooks-Walter,A., Tart,R.C., Briles,D.E. and Hollingshead,S.K.

TITLE Direct Submission

JOURNAL Submitted (26-SEP-1996) Microbiology, University of Alabama at Birmingham, 19th Street South, Box 10, Birmingham, AL 35294-2170, USA

FEATURES Location/Qualifiers

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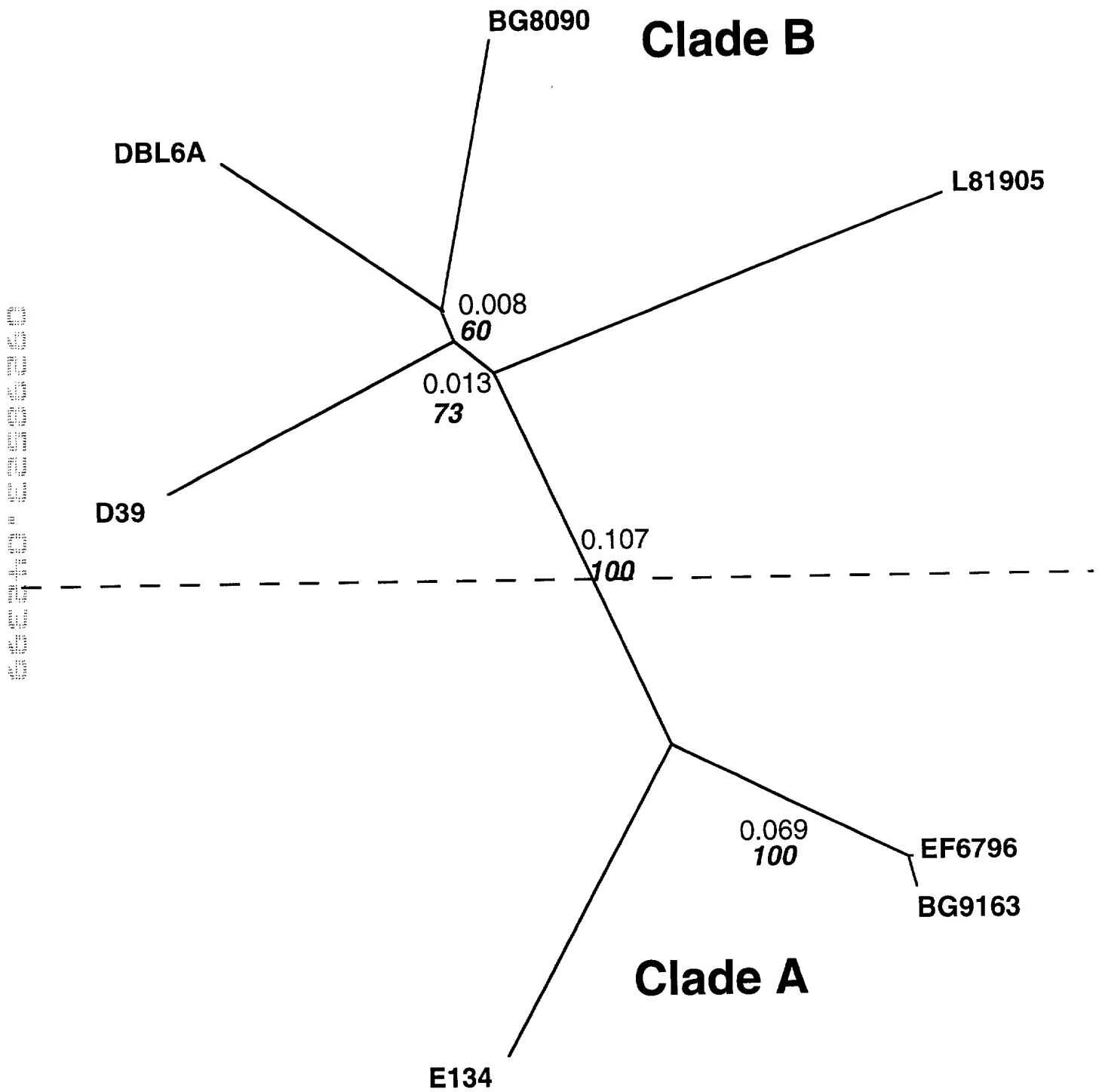
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 1981 gataccaaaa aagctaaact atcaaaactt gaagagttga gtgataagat tgatgagtta
 2041 gacgtgaaa ttgcaaaact tgaagtcaa cttaaagatg ctgaaggaaa caataatgta
 2101 gaagcctact ttaagaagg tttagagaaa actactgctg agaaaaaagc tgaattagaa
 2161 aaagctgaag ctgaccttaa gaaagcagtt gatgagccag aaactccagc tccggctcct
 2221 caaccagctc cagctccaga aaaaccagct gaaaaaccag ctccagctcc agaaaaacca
 2281 gctccagctc cagaaaaacc agctccagct ccagaaaaac cagctccagc tccagaaaaa
 2341 ccagctccag ctccagaaaa accagctcca actccagaaa ctccaaaaac aggcctggaaa
 2401 caagaaaacg gtatgtgcta ctctacaat actgatggtt caatggcaac aggcctggctc
 2461 caaaacaatg gctcatgcta ctacctcaac agcaatggcg ctatggcgac aggatggctc
 2521 caaaacaatg gctcatgcta ctacctcaac agcaatggcg ctatggcgac aggatggctc
 2581 caatacaatg gttcatgcta ctacctcaac gctaaggtg atatggcgac aggatggctc
 2641 caatacaatg gttcatgcta ctacctcaac gctaaggtg atatggcgac aggatggctc
 2701 caatacaatg gttcatgcta ctacctcaac gctaaggtg atatggcgac aggatggctc
 2761 caatacaatg gttcatgcta ctacctcaac gctaaggtg atatggcgac aggatggctc

2821 caatacaatg gttcatggta ctacctaaac agcaatgggtg ctatggtaac aggatggctc
2881 caaaacaatg gctcatggta ctacctaaac gctaacgggtt caatggcaac agattgggtg
2941 aaagatggag atacctggta ctatcttgaa gcatcaggtg ctatgaaagc aagccaatgg
3001 ttcaaagtat cagataaatg gtactatgtc aatggctcag gtgcccttgc agtcaacaca
3061 actgtagata gctatagagt caatgccaat ggtgaatggg taaactaaac ttaatataac
3121 tagttaatac tgacttcctg taagaactct ttaaagtatt ccctacaaat accatacct
3181 ttcagtagat aatataccct ttaggaagt ttagattaaa aaataactct gtaatctcta
3241 gccggattta tagcgctaga gactacggag ttttttgat gaggaaagaa tggcggcatt
3301 caagagactc ttaagagag ttacggggtt taaactatta agctttctcc aattgcaaga
3361 gggttcaat ctctgctagg tgctagcttg cgaaatggct ccacaggagt ttggcrgcgc
3421 cagatgttcc acggaggtag tgaggagcga ggccgcggaa ttc

//

the above report in format

FIG. 12



— 0.05 changes

Fig. 13

UAB PspC Sequences

EF6796	10	20	30	40	50	60	70
BG9163	MFAS	KSERK	YHYS	IRKFS	IGV	ASV	AVAS
E134	MFAS	KSERK	YHYS	IRKFS	IGV	ASV	AVAS
D39	MFAS	KSERK	YHYS	IRKFS	IGV	ASV	AVAS
DBL6A	MFAS	KSERK	YHYS	IRKFS	IGV	ASV	AVAS
BG8090	MFAS	KSERK	YHYS	IRKFS	IGV	ASV	AVAS
L81905	MFAS	KSERK	YHYS	IRKFS	IGV	ASV	AVAS
EF6796	80	90	100	110	120	130	140
BG9163	HL	ESIL	KD	YK	KN	LN	KK
E134	HL	ESIL	KD	YK	KN	LN	KK
D39	HL	ESIL	KD	YK	KN	LN	KK
DBL6A	HL	ESIL	KD	YK	KN	LN	KK
BG8090	HL	ESIL	KD	YK	KN	LN	KK
L81905	HL	ESIL	KD	YK	KN	LN	KK
EF6796	150	160	170	180	190	200	210
BG9163	ET	KE	KL	TA	TF	Q	IK
E134	ET	KE	KL	TA	TF	Q	IK
D39	ET	KE	KL	TA	TF	Q	IK
DBL6A	ET	KE	KL	TA	TF	Q	IK
BG8090	ET	KE	KL	TA	TF	Q	IK
L81905	ET	KE	KL	TA	TF	Q	IK
EF6796	220	230	240	250	260	270	280
BG9163	NY	PT	IT	TY	KT	LE	LE
E134	NY	PT	IT	TY	KT	LE	LE
D39	NY	PT	IT	TY	KT	LE	LE
DBL6A	NY	PT	IT	TY	KT	LE	LE
BG8090	NY	PT	IT	TY	KT	LE	LE
L81905	NY	PT	IT	TY	KT	LE	LE
EF6796	290	300	310	320	330	340	350
BG9163	EE	AK	RR	AD	AK	LL	KK
E134	EE	AK	RR	AD	AK	LL	KK
D39	EE	AK	RR	AD	AK	LL	KK
DBL6A	EE	AK	RR	AD	AK	LL	KK
BG8090	EE	AK	RR	AD	AK	LL	KK
L81905	EE	AK	RR	AD	AK	LL	KK

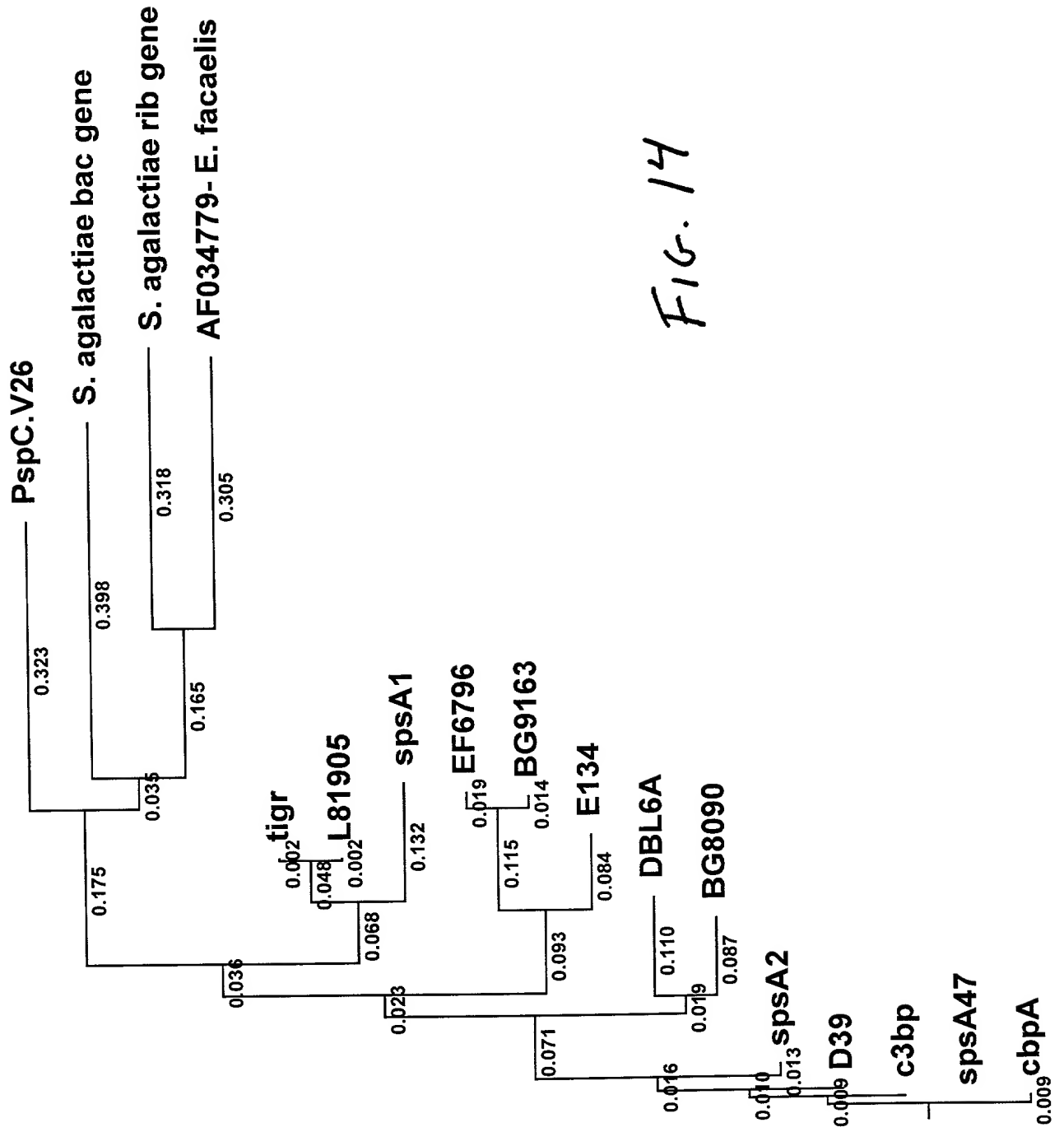
EF6796	SVGGEEITLPS	360	370	380	390	400	410	420
BG9163	SVGGEEITLPS	360	370	380	390	400	410	420
E134	SVGGEEITLPS	360	370	380	390	400	410	420
D39	SVGGEEITLPS	360	370	380	390	400	410	420
DBL6A	SVGGEEITLPS	360	370	380	390	400	410	420
BG8090	SVGGEEITLPS	360	370	380	390	400	410	420
LB1905	SVGGEEITLPS	360	370	380	390	400	410	420
EF6796	NSKKKEELTS	430	440	450	460	470	480	490
BG9163	NSKKKEELTS	430	440	450	460	470	480	490
E134	NSKKKEELTS	430	440	450	460	470	480	490
D39	NSKKKEELTS	430	440	450	460	470	480	490
DBL6A	NSKKKEELTS	430	440	450	460	470	480	490
BG8090	NSKKKEELTS	430	440	450	460	470	480	490
LB1905	NSKKKEELTS	430	440	450	460	470	480	490
EF6796	AESEBVKVKA	500	510	520	530	540	550	560
BG9163	AESEBVKVKA	500	510	520	530	540	550	560
E134	AESEBVKVKA	500	510	520	530	540	550	560
D39	AESEBVKVKA	500	510	520	530	540	550	560
DBL6A	AESEBVKVKA	500	510	520	530	540	550	560
BG8090	AESEBVKVKA	500	510	520	530	540	550	560
LB1905	AESEBVKVKA	500	510	520	530	540	550	560
EF6796	AEAKQKVDAE	570	580	590	600	610	620	630
BG9163	AEAKQKVDAE	570	580	590	600	610	620	630
E134	AEAKQKVDAE	570	580	590	600	610	620	630
D39	AEAKQKVDAE	570	580	590	600	610	620	630
DBL6A	AEAKQKVDAE	570	580	590	600	610	620	630
BG8090	AEAKQKVDAE	570	580	590	600	610	620	630
LB1905	AEAKQKVDAE	570	580	590	600	610	620	630
EF6796	DKIDELDAEI	640	650	660	670	680	690	700
BG9163	DKIDELDAEI	640	650	660	670	680	690	700
E134	DKIDELDAEI	640	650	660	670	680	690	700
D39	DKIDELDAEI	640	650	660	670	680	690	700
DBL6A	DKIDELDAEI	640	650	660	670	680	690	700
BG8090	DKIDELDAEI	640	650	660	670	680	690	700
LB1905	DKIDELDAEI	640	650	660	670	680	690	700

FIG. 13 (cont'd)

700 800 810 820 830 840

EF6796
BG9163
E134
D39
DBL6A
BG8090
LB1905

Fig 13 (cont'd)



LOCUS PSPC.V26 730 AA PROT SYN ~~30-10-2009~~

DEFINITION Streptococcus pneumoniae PspC protein from strain V26

ACCESSION -

KEYWORDS -

SOURCE -

FEATURES Location/Qualifiers

PROPEP <1..731

/note="3 to 2349 of V26.pspC (translated)"

ORIGIN -

1 MFASKSERKV HYSIRKFSIG VASVWVASLF LGGVWHAEV RRGNNLTVTS SGDEVESHYQ
61 SILEKVRKSL EKDRHTQNV D LIKKLQDIK TYLYNLKEP EAELTSKTNK ELDAAFEKFK
121 KEPELTKKLA EAEKKAKDQK EEDHRNYPTN TYKTIELEIA EAEVGVAKAE LELAQAQVQI
181 PQDTEKINAA KAKVEAAKSN VKKLEKIKSD IEKTYLYKLD NSTKETPKSR VRRNSPQVGD
241 SRELKETIDK AKETLSTYMV TRLTKLDPV FWFADLLMDA KKWVEEYKTK LEDASDKKSV
301 EDLRKEAEGK IESLIVTHQN REKENQPAPQ PGGQAGGSMV VPPVTQTTPS TSQSPGQKAT
361 EAEKKKLQDL IRQFQEALNK LDDETKTVPD GAKLTGEAGK AYNETRTRYAK EVVDKSKKLL
421 SQTAVTMDL AMQLTKLND MSKLKEAKAK LVPEVKPQPE NPEPKPQPEG EKPSVPDINQ
481 EKEKAKLAIA TYMSKILDDI KKHHLKKEKH HQIVALIKDL DKLRKQALSE IDNVNTKVEI
541 ENTVHKVFAD MDTVVTKFQK GLIQNTQPVP EAQRAQRYQR FQIHQKAPDT PQVPEAPKSP
601 EVPKVPEAPK APDTPQVPEA PKSPEVPKVS DTPKAPDTPQ VPEAPKSPEV PKVPEAPKAP
661 DTPQVPEAPK SPEVPKVPDT PKAPDTPQVP EAPKAPDTPQ IPEAPAPETP APAPEAPKTG
721 WKQENG MWKG

//

Fig. 15

Fig. 15 (cont'd)

LOCUS V26DNA.PSP 2349 BP SS-DNA SYN
DEFINITION Streptococcus pneumoniae PspC gene from strain V26
ACCESSION -
KEYWORDS -
SOURCE -
BASE COUNT 937 A 457 C 505 G 450 T 0 OTHER
ORIGIN -
1 AATTGGCCCT TCGACGAATA GCTGAAGAGG AAAAGCTATT ACATGAAGTT ATAATCCCAA
61 ATGGAAGCAT AAAGAGATAA ATACAAAATT CGATTTATAT ACAGTTTCATA TTGAAGTGAT
121 ATAGTAAGGC TAAAGAAAAA ATATAGAAGG AAATAAACAT GTTTGCATCA AAAAGCGAAA
181 GAAAAGTACA TTATTCAATT CGTAAATTTA GTATTGGAGT AGCTAGTGTA GTAGTTGCTA
241 GTTTGTTCTT AGGAGGAGTA GTTCACGCGA AAGAGGTTAG AAGAGGGAAT AACCTCACGG
301 TTACATCTAG TGGGGATGAA GTCGAGTCGC ATTATCAAAG TATATTGGAG AAGGTCAGAA
361 AAAGTTTGGG AAAAGATCGA CATACCCAAA ATGTCGACTT ATCAAAAAG TTGCAAGACA
421 TTAAGAGAAC GTATTGTAT AATTTAAAG AGAAGCCGGA AGCTGAGTTG ACGTCAAAA
481 CAAATAAAGA GTTAGACGCA GCTTTTGAGA AGTTTAAAA AGAACCGAA CTTACTAAAA
541 AATTAGCAGA AGCTGAGAAA AAGCCCAAGG ATCAAAAAGA AGAAGATCAC CGTAACTACC
601 CAACCAATAC TTACAAAACA ATCGAATGGA AATTGGGA AGCAAGTA GGGGTCGCCA
661 AGGCAGAGCT TGAGCTTGCA CAAGCTCAAG TCCAATACC TCAAGATACT GAGAAAATTA
721 ATGCTGCTAA AGCTAAAGTA GAAGCTGCTA AAGTAATGT TAAAAACTA GAAAAAATTA
781 AATCAGATAT TGAATAAACG TATTTGATA AATTAGATAA CTCAACCAA GAAACGCCAA
841 AATCTAGAGT GCGAAGAAAT TCTCCGCAAG TAGGCGATTG GAGAGAACTT AAGGAAACGA
901 TAGACAAAGC GAAAGAACT CTGTCTACCT ATATGGTAA TCGTTTAAAC AAGCTGGATC
961 CATCTGTTT TTGGTTTGCA GATCTTCTTA TGGATGCTAA GAAAGTTGTG GAAGAATACA
1021 AGACAAAATT AGAGGATGCT TCAGATAAAA AATCGGTAGA AGACTTGCGA AAGGAAGCAG
1081 AAGGAAAAT AGAGTCTCTT ATCGTGACTC ACCAAATAG AGAAAAAGAA AACCAACCAG
1141 CACCCCAACC AGGAGGACAA GCAGGTGGT CAATGGTTGT ACCACCGGTG ACGCAAACAC
1201 CTCCATCAAC TTCCCAAAGT CCAGGACAAA AGGCGACCGA AGCTGAAAAA AAAAAAGTTAC
1261 AAGACTTGAT TCGTCAATC CAAGAAGCCT TGAACAACT AGACGATGAA ACAAAGACTG
1321 TTCAGATGG GGCTAACTC ACAGGAGAAG CTGAAAAAGC CTATAATGAG ACTAGAACTT
1381 ATGCGAAAGA AGTTGTTGAC AAGAGCAAGA AGCTTCTATC ACAGACAGCA GTGACAAATGG
1441 ATGAATTGGC AATGCAATTA ACCAAATTGA ACGATGCCAT GTCTAAATTG AAAGAAGCTA
1501 AAGCGAATT GGTACCAGAG GTTAAACCCAC AGCCGGAAAA CCCAGAGCCA AACCCACAAC
1561 CAGAGGGTGA GAAACCAAGC GTACCAGATA TTAATCAGGA GAAAGAAAAA GCTAAACTTG
1621 CTATAGCAAC ATACATGAGC AAGATTTTAG ATGATATAAA GAAACATCAT CTGAAGAAAG
1681 AAAACATCA TCAGATTGTT GCTCTTATTA AGGACCTTGA TAACTTAGA AAGCAAGCAC
1741 TTTCTGAAT TGATAATGTA AATACCAAAG TAGAAATTGA GAATACAGTC CACAAGGTAT
1801 TTGCAGACAT GGATACGGTT GTTACTAAAT TCCAAAAAGG CTTAATTTCAG AACACACCGC
1861 AGGTTCCAGA AGCCCAAAGA GCCCAGAGGT ACCAAAGGTT TCAGATACAC CAAAAGGCTC

1921 CGGACACACC GCAGGTTCCA GAAGCACCAA AGAGCCCAGA GGTACCAAAG GTTCCAGAAG
1981 CACCAAAGGC TCCGGACACA CCGCAAGTTC CGGAAGCACC AAAGAGCCCA GAGGTACCAA
2041 AGGTTTCAGA TACACCAAAG GCTCCGGACA CACCGCAGGT TCCAGAAGCA CCAAAGAGCC
2101 CAGAGGTACC AAAGGTTCCA GAAGCACCAA AGGCTCCGGA CACACCGCAA GTTCCGGAAG
2161 CACCAAAGAG CCCAGAGGTA CCAAAGGTTT CAGATACACC AAAGGCTCCG GACACACCGC
2221 AGGTTCCAGA AGCACCAAAG GCTCCAGACA CACCGCAAAT TCCGGAAGCA CCAGCTCCAG
2281 AAAC TCCGGC TCCAGCTCCA GAAGCTCCA AAACAGGCTG GAAACAAGAA AACGGTATGT
2341 GGAAGGGCG

//

Fig. 15 (cont'd)

Fig. 16

LOCUS E134.DNA 2405 BP SS-DNA SYN ~~XXXXXXXXXX~~
DEFINITION -
ACCESSION -
KEYWORDS -
SOURCE -
FEATURES Location/Qualifiers
CDS 187..2405
BASE COUNT 1046 A 386 C 505 G 468 T 0 OTHER
ORIGIN -

1 CGGCCGCCAG TGTGATGGAT ATCTGCAGAA TTCGCCCTTC GACGAATAGC TGAAGAGGAA
61 AAGCTATTAC ATGAAGTTAT AATCCCAAAT GGAAGCATAA AGAGATAAAT ACAAATTTCG
121 ATTTATATAC AGTTCATATT GAAGTGATAT AGTAAGGTTA AAGAAAAAAT ATAGAAGGAA
181 ATAAACATGT TTGCATCAAA AAGCGAAAGA AAAGTACATT ATTCAATTCG TAAATTTAGT
241 ATTGGAGTAG CTAGTGTAGT AGTTGCTAGT CTTGTTATGG GAAGTGTGGT TCATGCGACG
301 GAGAATGAGG GAATTACCCA AGTAGCCACT TCTTATAATA AGGCAAATGA AAGTCAGACA
361 GAACATAGGA AAGCTGCTAA ACAAGTCGAT GAAGATATAA AAAAAATGTT GAGTGAGATC
421 CAAGAATATA TAAAAAAAT GTTGAGTGAG ATCCAATTAG ATAAAAGAAA ACATACCCAA
481 AATGTCAACT TAAACAGAAA GTTGAGCGCA ATTCAAACGA AGTATTTGTA TGAATTAAGA
541 GTTTTAAAAG AGAAGTCGAA AAAAGAAGAG TTGACGTCAA AAACAAAAAA AGAGTTAGAC
601 GCAGCTTTTG AGAAGTTTAA AAAAGAACCA GAACTTACTA AAAAATTAGC AGAAGCTAAA
661 CAAAAGCCA AGGCTCAAAA AGAAGAAGAT TTCCGTAAC TACCAACCAA TACTTACAAA
721 ACGCTTGAAC TTGAAATTGC TGAGTTCGAT GTGAAAGTTA AAGAAGCGGA GCTTGAAC TA
781 GTAAAAGAGG AAGCTAAACC CCGAAACGAG GAAAAATTA AGCAAGCAAA AGCGAAAGTT
841 GAGAGTAAAA AAGCTGAGGC TACAAGGTTA GAAGAAATCA AGACAGAACG TAAAAAGCA
901 GAAGAAGAAG CTAACGAAA AGCAGAAGAA TCTGAGAAAA AAGCTGCTGA AGCCAAACAA
961 AAAGTGGATA CTAAGAGCA AGGTAAACCA AAGAGGCGGG CAAAACGAGG

AGTTTCTGGA

1021 GAGCTAGCAA CACCTGATAA AAAAGAAAAT GATGCGAAGT CTTAGATTTC TAGCGTAGGT
1081 GAAGAACTC TTCCAAGCCC ATCCCTTAAT ATGGCAAATG AAAGTCAGAC AGAACATAGG
1141 AAAGATGTCG ATGAATATAT AAAAAAATG TTGAGTGAGA TCCAATTAGA TAGAAGAAAA
1201 CATACCCAAA ATGTCAACTT AAACATAAAG TTGAGCGCAA TTAACCGAA GTATTTGTAT
1261 GAATTAAGTG TTTTAAAAGA GAACTCGAAA AAAGAAGAGT TGACGTCAA AACCAGCA
1321 GAGTTAACCG CAGCTTTTGA GCAGTTTAAA AAAGATACAT TGAACCCAGA AAAAAAGGTA
1381 GCAGAAGCTG AGAAGAAGGT TGAAGAAGCT AAGAAAAAAG CCAAGGATCA

AAAAGAAGAA

1441 GATCGCCGTA ACTACCCAAC CAATACTTAC AAAACGCTTG AACTTGAAAT TGCTGAGTCC
1501 GATGTGAAAG TTAATAAAGC GGAGCTTGAA CTAGTAAAAG AGGAAGCTAA CGAATCTCGA
1561 AACGAGGAAA AAATTAAGCA AGCAAAAGAG AAAGTTGAGA GTAAAAAAGC TGAGGCTACA
1621 AGGTTAGAAA AAATCAAGAC AGATCGTAAA AAAGCAGAAG AAGAAGCTAA ACGAAAAGCA
1681 GAAGAATCTG AGAAAAAAGC TGCTGAAGCC AAACAAAAAG TGGATGCTGA AGAATATGCT
1741 CTTGAAGCTA AAATCGCTGA GTTGGAATAT GAAGTTCAGA GACTAGAAAA AGAGCTCAAA
1801 GAGATTGATG AGTCTGACTC AGAAGATTAT CTTAAAGAAG GCCTCCGTGC TCCTCTTCAA
1861 TCTAAATTGG ATACCAAAAA AGCTAACTA TCAAACTTG AAGAGTTGAG TGATAAGATT
1921 GATGAGTTAG ACGCTGAAAT TGCAAACTT GAAGTTCAAC TTAAGATGC TGAAGGAAAC
1981 AATAATGTAG AAGCCTACTT TAAAGAAGGT TTAGAGAAAA CACTGCTGA GAAAAAGCT
2041 GAATTAGAAA AAGCTGAAGC TGACCTTAAG AAAGCAGTTG ATGAGCCAGA AACTCCAGCT
2101 CCGGCTCCTC AACCAGCTCC AGCTCCAGAA AAACCAGCTG AAAAACCAGC

TCCAGCTCCA

2161 GCTCCAGAAA AACCAGCTCC AGCTCCAGAA AAACCAGCTG AAAAACCAGC

TGAAAAACCA

2221 GCTGAAGAAC CAGCTGAAAA ACCAGCTCCA GCTCCAGAAA AACCAGCTCC

AACTCCAGAA

2281 AAACCAGCTC CAACTCCAGA AACTCCAAAA ACAGGCTGGA AACAAGAAAA


CGGTATGTGG

2341 TACTTCTACA ATACTGATGG TTCAATGGCA ACAGGCTGGC TCCAAAACAA TGGTTCATGG
2401 TACTA

//

Fig. 16 (cont'd)

Fig. 17

LOCUS D39.DNA 2774 BP SS-DNA SYN 
DEFINITION -
ACCESSION -
KEYWORDS -
SOURCE -
FEATURES Location/Qualifiers
CDS 362..2467
BASE COUNT 1057 A 476 C 615 G 624 T 2 OTHER
ORIGIN -

1 CCAAGCTATT AGGTGACACT ATAGAATACT CAAGCTATGC ATCAAGCTTA TGCTTGTCAA
61 TAATCACAAA TATGTAGATC ATATCTTGTT TAGGACAGTA AACATCCTA ATTACTTTTT
121 AAATATTCTT CCTGAGTTGA TTGGCTTGAC CTTGTTGAGT CATGCTTATG TGACTTTTGT
181 TTTAGTTTTT CCAGTTTATG CAGTTATTTT GTATCGACGA ATAGCTGAAG AGGAAAAGCT
241 ATTACATGAA GTTATAATCC CAAATGGAAG CATAAAGAGA TAAATACAAA ATTCGATTTA
301 TATACAGTTC ATATTGAAGT AATATAGTAA GGTAAAGAA AAAATATAGA AGGAAATAAA
361 CATGTTTGCA TCAAAAAGCG AAAGAAAAGT ACATTATTCA ATTCGTAAAT TTAGTATTGG
421 AGTAGCTAGT GTAGCTGTTG CCAGTCTTGT TATGGGAAGT GTGGTTCATG CGACAGAGAA
481 CGAGGGAAGT ACCCAAGCAG CCACTTCTTC TAATATGGCA AAGACAGAAC ATAGGAAAGC
541 TGCTAAACAA GTCGTCGATG AATATATAGA AAAAATGTTG AGGGAGATTC AACTAGATAG
601 AAGAAAACAT ACCCAAATG TCGCCTTAAA CATAAAGTTG AGCGCAATTA AAACGAAGTA
661 TTTGCGTGAA TTAAATGTTT TAGAAGAGAA GTCGAAAGAT GAGTTGCCGT CAGAAATAAA
721 AGCAAAGTTA GACGCAGCTT TTGAGAAGTT TAAAAAGAT ACATTGAAAC CAGGAGAAAA
781 GGTAGCAGAA GCTAAGAAGA AGGTTGAAGA AGCTAAGAAA AAAGCCGAGG ATCAAAAAGA
841 AGAAGATCGT CGTAACTACC CAACCAATAC TTACAAAACG CTTGAACTTG AAATTGCTGA
901 GTTCGATGTG AAAGTTAAAG AAGCGGAGCT TGAAGTAGTA AAAGAGGAAG CTAAAGAATC
961 TCGAAACGAG GGCACAATTA AGCAAGCAAA AGAGAAAGTT GAGAGTAAAA AAGCTGAGGC
1021 TACAAGGTTA GAAAACATCA AGACAGATCG TAAAAAGCA GAAGAAGAAG CTAAACGAAA
1081 AGCAGATGGT AAGTTGAAGG AAGCTAATGT AGCGACTTCA GATCAAGGTA
AACCAAAGGG
1141 GCGGGCAAAA CGAGGAGTTC CTGGAGAGCT AGCAACACCT GATAAAAAAG
AAAATGATGC
1201 GAAGTCTTCA GATTCTAGCG TAGGTGAAGA AACTCTTCCA AGCTCATCCC TGAAATCAGG
1261 AAAAAAGGTA GCAGAAGCTG AGAAGAAGGT TGAAGAAGCT GAGAAAAAAG
CCAAGGATCA
1321 AAAAGAAGAA GATCGCCGTA ACTACCCAAC CAATACTTAC AAAACGCTTG ACCTTGAAAT
1381 TGCTGAGTCC GATGTGAAAG TTAAAGAAGC GGAGCTTGAA CTAGTAAAAG
AGGAAGCTAA
1441 GGAACCTCGA GACGAGGAAA AAATTAAGCA AGCAAAAGCG AAAGTTGAGA
GTAAAAAGC
1501 TGAGGCTACA AGGTTAGAAA ACATCAAGAC AGATCGTAAA AAAGCAGAAG AAGAAGCTAA
1561 ACGAAAAGCA GCAGAAGAAG ATAAAGTTAA AGAAAAACCA GCTGAACAAC CACAACCAGC
1621 GCCGGCTACT CAACCAGAAA AACCAGCTCC AAAACCAGAG AAGCCAGCTG
AACAACCAAA
1681 AGCAGAAAAA ACAGATGATC AACAAGCTGA AGAAGACTAT GTCGTTAGAT CAGAAGAAGA
1741 ATATAATCGC TTGACTCAAC AGCAACCGCC AAAAAGTGA AAACCAGCAC AACCATCTAC
1801 TCAAAAACA GGCTGGAAAC AAGAAAACGG TATGTGGTAC TTCTACAATA CTGATGGTTC
1861 AATGGCAACA GGATGGCTCC AAAACAACGG TTCATGGTAC TATCTAAACG CTAATGGTGC
1921 TATGGCGACA GGATGGCTCC AAAACAATGG TTCATGGTAC TATCTAAACG CTAATGGTTC
1981 AATGGCAACA GGATGGCTCC AAAACAATGG TTCATGGTAC TACCTAAACG CTAATGGTGC
2041 TATGGCGACA GGATGGCTCC AATACAATGG TTCATGGTAC TACCTAAACA
GCAATGGCGC
2101 TATGGCGACA GGATGGCTCC AATACAATGG CTCATGGTAC TACCTCAACG
CTAATGGTGA
2161 TATGGCGACA GGATGGCTCC AAAACAACGG TTCATGGTAC TACCTCAACG

CTAATGGTGA

2221 TATGGCGACA GGATGGCTCC AATACAACGG TTCATGGTAT TACCTCAACG CTAATGGTGA

2281 TATGGCGACA GGTTGGGTGA AAGATGGANA TACCTGGTAC TATCTTAAAG

CATCAGGTGC

2341 TATGAAAGCA AGCCAATGGT TCAAAGTATC AGATAAATGG TACTATGTCA ATGGCTCAGG

2401 TGCCCTTGCA GTCAACACAA CTGTAGATGG CTATGGAGTC AATGCCAATG

GTGAATGGGT

2461 AAATAAACC TAATATAACT AGTTAATACT GACTTCCTGT AAGAACTTTT TAAAGTATTC

2521 CCTACAAATA CCATATCCTT TCAGTAGATA ATATACCCTT GTAGGAAGTT TAGATTAAAA

2581 AATAACTCTG TAATCTCTAG CCGGATTTAT AGCGCTAGAG ACTACGGAGT TTTTTTGATG

2641 AGGAAAGAAT GCGGCGATTG AAGAGACTCT TTAAGAGAGT TACGGGTTTT AAATAATTAA


2701 GCCTTCTCCA ATTGCAAGAG GGCTTCAATC TCTGCTAGGG TGCTAGCTTG

CGAAATGGCT

2761 CCACGGAGTT TNGC

//

Fig. 17 (cont'd)

LOCUS BG9163.DNA 2489 BP SS-DNA SYN  *Fig. 18*
DEFINITION -
ACCESSION -
KEYWORDS -
SOURCE -
FEATURES Location/Qualifiers
CDS 221..2489
BASE COUNT 1026 A 429 C 541 G 493 T 0 OTHER
ORIGIN -

1 GATTGTATAC GACCACTATA GGGCGAATTG GGCCCGACGT CGCATGCTCC CGGCCGCCAT
61 GGCCGCGGGT ATTCGACGAA TAGCTGAAGA GGAAAAGCTA TTACATGAAG TTATAATCCC
121 AAATGGAAGC ATAAAGAGAT AAATACAACA TTCGATTTAT ATACAGTTCC TATTGAAGTG
181 ATATAATAAG GTTAAAGAAA AAATATAGAA GGAAATAAAC ATGTTTGCAT CAAAAAGCGA
241 AAGAAAAGTA CATTATTCAA TTCGTAAATT TAGTATTGGA GTAGCTAGTG TAGCTGTTGC
301 CAGCTTGTTT TTAGGAGGAG TAGTCCATGC AGAAGGGGTT AGAAGTGGGA ATAACCTCAC
361 GGTACATCT AGTGGGCAAG ATATATCGAA GAAGTATGCT GATGAAGTCG AGTCGCATCT
421 AGAAAGTATA TTGAAGGATG TCAAAAAAAA TTTGAAAAA GTTCAACATA CCCAAATGT
481 CGGCTTAATT ACAAAGTTGA GCGAAATTAA AAAGAAAGTAT TTGTATGACT TAAAAGTTAA
541 TGTTTTATCG GAAGCTGAGT TGACGTCAAA AACAAAAGAA ACAAAGAAA AGTTAACCGC
601 AACTTTTGAG CAGTTTAAAA AAGATACATT ACCAACAGAA CCAGAAAAAA AGGTAGCAGA
661 AGCTCAGAAG AAGGTTGAAG AAGCTAAGAA AAAAGCCGAG GATCAAAAAG AAAAGATCG
721 CCGTAACTAC CCAACCATA CTTACAAAAC GCTTGAACCT GAAATTGCTG AGTCCGATGT
781 GGAAGTTAAA AAAGCGGAGC TTGAACTAGT AAAAGTGAAA GCTAAGGAAT CTCAAGACGA
841 GGAAAAAATT AAGCAAGCAG AAGCGGAAGT TGAGAGTAAA CAAGCTGAGG CTACAAGGTT
901 AAAAAAATC AAGACAGATC GTGAAGAAGC TAAACGAAAA GCAGATGCTA AGTTGAAGGA
961 AGCTGTTGAA AAGAATGTAG CGACTTCAGA GCAAGATAAA CCAAAGAGGC
GGGCAAAACG
1021 AGGAGTTTCT GGAGAGCTAG CAACACCTGA TAAAAAGAA AATGATGCGA AGTCTTCAGA
1081 TTCTAGCGTA GGTGAAGAAA CTCTTCCAAG CCCATCCCTT AATATGGCAA ATGAAAGTCA
1141 GACAGAACAT AGGAAAGATG TCGATGAATA TATAAAAAAA ATGTTGAGTG AGATCCAATT
1201 AGATGGAAGA AACATACCC CAAATGTCAA CTTAAACATA AAGTTGAGCG CAATTTAAAC
1261 GAAGTATTTG TATGAATTAA GTGTTTAAA AGAGAACTCG AAAAAAGAAG AGTTGACGTC
1321 AAAAACCAAA GCAGAGTTAA CCGCAGCTTT TGAGCAGTTT AAAAAAGATA CATTGAAACC
1381 AGAAAAAATA GTAGCAGAAG CTGAGAAGAA GGTGAAGAA GCTAAGAAAA
AAGCCAAGGA
1441 TCAAAAAGAA GAAGATCGCC GTAACACCC AACCAATACT TACAAAACGC TTGAACTTGA
1501 AATTGCTGAG TCCGATGTGA AAGTTAAAGA AGCGGAGCTT GAACTAGTAA
AAGAGGAAGC
1561 TAACGAATCT CGAAACGAGG AAAAAATTAA GCAAGCAAAA GAGAAAGTTG AGAGTAAAAA
1621 AGCTGAGGCT ACAAGGTTAG AAAAAATCAA GACAGATCGT AAAAAAGCAG AAGAAGAAGC
1681 TAAACGAAAA GCAGAAGAAT CTGAGAAAAA AGCTGCTGAA GCCAAACAAA AAGTGGATGC
1741 TGAAGAATAT GCTCTTGAAG CTAAAATCGC TGAGTTGGAA TATGAAGTTC AGAGACTAGA
1801 AAAAGAGCTC AAAGAGATTG ATGAGTCTGA CTCAGAAGAT TATCTTAAAG AAGGCCTCCG
1861 TGCTCCTCTT CAATCTAAAT TGGATACCAA AAAAGCTAAA CTATCAAAAC TTGAAGAGTT
1921 GAGTGATAAG ATTGATGAGT TAGACGCTGA AATTGCAAAA CTTGAAGTTC AACTTAAAGA
1981 TGCTGAAGGA AACAATAATG TAGAAGCCTA CTTTAAAGAA GGTTTAGAGA AACTACTGTC
2041 TGAGAAAAAA GCTGAATTAG AAAAAGCTGA AGCTGACCTT AAGAAAGCAG TTGATGAGCC
2101 AGAAACTCCA GCTCCGGCTC CTCAACCAGC TCCGGCTCCA GAAAAACCAG
CTGAAAAACC
2161 AGCTCCAGCT CCAGCTCCAG AAAAACCAGC TCCAGCTCCA GAAAAACCAG
CTCCAGCTCC
2221 AGAAAAACCA GCTCCAGCTC CAGAAAAACC AGCTCCAGCT CCAGAAAAAC
CAGCTCCAGC
2281 TCCAGAAAAA CCAGCTCCAG CTCCAGAAAA ACCAGCTCCA GCTCCTAAAC CAGAACTCC

2341 AGAAACAGGC TGGAAACAAG AAAACGGTAT GTGGTACTTC TACAATACTG ATGGTTCAAT
2401 GGCAACAGGC TGGCTCCAAA ACAATGGCTC ATGGTACTAC CTCAACAGCA
ATGGCGTTAT
2461 GGCGACAGGA TGGTTCCCAA ACAATGGTC
//

Fig. 18 (cont'd)


LOCUS BG8090.DNA 1680 BP SS-DNA SYN 
DEFINITION -
ACCESSION -
KEYWORDS -
SOURCE -
FEATURES Location/Qualifiers
CDS 220..1680
BASE COUNT 714 A 287 C 375 G 304 T 0 OTHER
ORIGIN -

Fig. 19

```
1 ATTGTATACG ACTCACTATA GGGCGAATTG GGCCCGACGT CGCATGCTCC CGGCCGCCAT
61 GGCCGCGGGA TTCGACGAAT AGCTGAAGAG GAAAAGCTAT TACATGAAGT TATAATCCCA
121 AATGGAAGCA TAAAGAGATA AATACAAAAT TCGATTTATA TACAGTTCAT ATTGAAGTGA
181 TATAGTAAGG TTAAAGAAAA AATATAGAAG GAAATAAACA TGTTTGCATC AAAAAACGAA
241 AGAAAAGTAC ATTATTCAAT TCGTAAATTT AGTATTGGAG TAGCTAGTGT AGCTGTTGCC
301 AGTCTTTTTTA TGGGAAGTGT GGTTTCATGCG ACAGAGAAGG AGGTAAGTAC CCAAGTAGCC
361 ACTTCTTTTAA ATAAGGCAAA TAAAAGTCAG ACAGAACATA TGAAAGCTGC TAAACAAGTC
421 GATGAATATA TAACAAAAAA GCTCCAATTA GATAGAAGAA AACATACCCA AAATGTGCGC
481 TTAATCACAAG AGTTGGGCGT AATTAAACG GAGTATTTGC ATAGATTAAG TGTTTCAAAA
541 GAGAAGTCGG AAGCTGAGTT GCCGTCAGAA ATAAAAGCAA AGTTAGACGC AGCTTTTGAG
601 CAGTTTAAAA AAGATACATT ACCAACAGAA CCAGGAAAAA AGGTAGCAGA AGCTGAGAAG
661 AAGGTTGAAG AAGCTAAGAA AAAAGCCGAG GATCAAAAAG AAGAAGATCG TCGTAACTAC
721 CCAACCATTA CTTACAAAAC GCTTGAACCT GAAATTGCTG AGTCCGATGT GGAAGTTAAA
781 AAAGCGGAGC TTGAACTAGT AAAAGAGGAA GCTAAGGGAT CTCGAAACGA GCAAAAAGTT
841 AACCAGCAA AAGCGAAAGT TGAGAGTAAA CAAGCTGAGG CTACAAGGT AAAAAAATC
901 AAGACAGATC GTGAACAAGC TGAGACTACA AGGTTAGAAA ACATCAAGAC AGATCGTGAA
961 AAAGCAGAAG AAGCTAAACG AAAAGCAGAT GCTAAAGAGC AAGATGAATC
AAAGAGGCGG
1021 GTAAAAGGAG GAGTTCCGGG AGAGCAAGCA ACACTTGATA AAAAAGAAAA
TGATGCGAAG
1081 TCTTCAGATT CTAGCGTAGG TGAAGAACT CTTCCAAGCC CATCCCTGAA ATCAGGAAAA
1141 AAGGTAGCAG AAGCTGAGAA GAAGGTTGCA GAAGCTGAGA AAAAAGCCAA
GGATCAAAAA
1201 GAAGAAGATC GCCGTAAC TAACCAACCAAT ACTTACAAAA CGCTTGAAC TGAATTGCT
1261 GAGTCCGATG TGAAAGTTAA AGAAGCGGAG CTTGAACTAG TAAAAGAGGA
AGCTAAGGAA
1321 TCTCGAAACG AGGAAAAAGT TAAGCAAGCA AAAGCGGAAG TTGAGAGTAA
AAAAGCTGAG
1381 GCTACAAGGT TAGAAAAAAT CAAGACAGAT CGTAAAAAAG CAGAAGAAGC TAAACGAAAA
1441 GCAGCAGAAG AAGATAAAGT TAAAGAAAAA CCAGCTGAAC AACCACAACC
AGCGCCGGCT
1501 CCTCAACCAG AAAAACCAGC TCCAGCTCCA AAACCAGAGA ATCCAGCTGA ACAACCAAAA
1561 GCAGAAAAAC CAGCTGATCA ACAAGCTGAA GAAGACTATG CTCGTAGATC AGAAGAAGAA
1621 TATAATCGCT TGAATCAACA GCAACCGCCA AAAACTGAAA AACCAGCACA ACCATCTACT
```

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LOCUS L81905.DNA 1766 BP SS-DNA SYN 
DEFINITION -
ACCESSION -
KEYWORDS -
SOURCE -
FEATURES Location/Qualifiers
CDS 217..1766
BASE COUNT 741 A 313 C 402 G 309 T 1 OTHER
ORIGIN -

Fig. 20

1 GTATACGACT CACTATAGGG CGAATTGGGC CCGACGTCGC ATGCTCCCGG
CCGCCATGGC

61 CGCGGGATTG GACGAATAGC TGAAGAGGAA AAGCTATTAC ATGAAGTTAT AATCCCAAAT
121 GGAAGCATAA AGAGATAAAT ACAAATTTCG ATTTATATAC AGTTCATATT GAAGTGATAT
181 AGTAAGGTTA AAGAAAAAAT ATAGAAGGAA ATAAACATGT TTGCATCAAA AAGCGAAAGA
241 AAAGTACATT ATTCAATTTCG TAAATTTAGT GTTGGAGTAG CTAGTGTAGT TGTTGCCAGT
301 CTTGTTATGG GAAGTGTGGT TCATGCGACA GAGAACGAGG GAGCTACCCA

AGTACCCAAT

361 TCTTCTAATA GGGCAAATGA AAGTCAGGCA GAACAAGGAG AACAACCTAA AAAACTCGAT
421 TCAGAACGAG ATAAGGCAAG GAAAGAGGTC GAGGAATATG TAAAAAAAT AGTGGGTGAG
481 AGCTATGCAA AATCAACTAA AAAGCGACAT ACAATTACTG TAGCTCTAGT TAACGAGTTG
541 AACAACATTA AGAACGAGTA TTTGAATAAA ATAGTTGAAT CAACCTCAGA AAGCCAACTA
601 CAGATACTGA TGATGGAGAG TCGATCAAAA GTAGATGAAG CTGTGTCTAA GTTTGAAAAG
661 GACTCATCTT CTTCGTCAAG TTCAGACTCT TCCACTAAAC CGGAAGCTTC AGATACAGCG
721 AAGCCAAACA AGCCGACAGA ACCAGGAGAA AAGGTAGCAG AAGCTAAGAA

GAAGGTTGAA

781 GAAGCTGAGA AAAAAGCCAA GGATCAAAAA GAAGAAGATC GTCGTAACCTA CCCAACCATT
841 ACTTACAAAA CGCTTGAAC TGAATTGCT GAGTCCGATG TGGAAGTTAA AAAAGCGGAG
901 CTTGAACTAG TAAAGTGAA AGCTAACGAA CCTCGAGACG AGCAAAAAAT TAAGCAAGCA
961 GAAGCGGAAG TTGAGAGTAA ACAAGCTGAG GCTACAAGGT TAAAAAAAT CAAGACAGAT
1021 CGTGAAGAAG CAGAAGAAGA AGCTAAACGA AGAGCAGATG CTAAAGAGCA

AGGTAAACCA

1081 AAGGGGCGGG CAAAACGAGG AGTTCCTGGA GAGCTAGCAA CACCTGATAA

AAAAGAAAAT

1141 GATGCGAAGT CTTCAAGATTC TAGCGTAGGT GAAGAACTC TTCCAAGCCC ATCCCTGAAA
1201 CCAGAAAAAA AGGTAGCAGA AGCTGAGAAG AAGGTTGAAG AAGCTAAGAA

AAAAGCCGAG

1261 GATCAAAAAG AAGAAGATCG CCGTAACTAC CCAACCAATA CTTACAAAAC GCTTGAACCT
1321 GAAATTGCTG AGTCCGATGT GGAAGTTAAA AAAGCGGAGC TTGAACTAGT AAAAGAGGAA
1381 GCTAAGGAAC CTCGAAACGA GGAAAAAGTT AAGCAAGCAA AAGCGGAAGT

TGAGAGTAAA

1441 AAAGCTGAGG CTACTAGGTT AGAAAAATC AAGACAGATC GTAAAAAAGC AGAAGAAGAA
1501 GCTAAACGAA AAGCAGCAGA AGAAGATAAA GTTAAAGAAA AACCAGCTGA ACAACCACAA
1561 CCAGCGCCGG CTCCAAAAGC AGAAAAACCA GCTCCAGCTC CAAAACCAGA

GAATCCAGCT

1621 GAACAACCAA AAGCAGAAAA ACCAGCTGAT CAACAAGCTG AAGAAGAGTA TGCTCGTAGA
1681 TCAGAAGAAG AATATAATCG CTTGACTCTA CAGCAACCGC CAAAACTGA AAAACCAGCA
1741 CAACCATCTA CTCCAAAAC AAANAC

//

LOCUS DBL6A.DNA 1590 BP SS-DNA SYN ~~XXXXXXXXXX~~
DEFINITION -
ACCESSION -
KEYWORDS -
SOURCE -
FEATURES Location/Qualifiers
CDS 127..1590
BASE COUNT 701 A 261 C 339 G 288 T 1 OTHER
ORIGIN -

Fig. 21

1 AAACATTAC ATGAAGTTAT AATCCCAAAT GGAAGCATAA AGAGATAAAT ACAAATTCG
61 ATTTATATAC AGTTCATATT GAAGTGATAT AGTAAGGTTA AAGAAAAAAT ATAGAAGGAA
121 ATAATTATGT TTGCATCYAA AAGCGAAAGA AAAGTACATT ATTCAATTCG TAAATTTAGT
181 ATTGGAGTAG CTAGTGTAGC TGTTGCTAGC TTGTTCTTAG GAGGAGTAGT CCATGCAGAA
241 GGGGTTAGAA GTGAGAATAC CCCCAAGGTT ACATCTAGTG GGGATGAAGT CGATGAATAT
301 ATAAAAAATA TGTTGAGTGA GATCCAATTA GATAAAAGAA AACATACCCA CAATTTGCGC
361 TTAAACCTAA AGTTGAGCAG AATTAAAACG GAGTATTTGT ATAAATTAAA AGTTAATGTT
421 TTAGAAGAAA AGTCAAAAGC TGAGTTGACG TCAAAAACAA AAAAAGAGGT AGACGCAGCT
481 TTTGAGAAGT TAAAAAAGA TACATTGAAA CTAGGAGAAA AGGTAGCAGA AGCTCAGAAG
541 AAGGTTGAAG AAGCTAAGAA AAAAGCCAAG GATCAAAAAG AAGAAGATCA CCGTAACTAC
601 CCAACCAATA CTTACAAAAC GCTTGAACCTT GAAATTGCTG AGTCCGATGT GAAAGTTAAA
661 GAAGCGGAGC TTGAACCTATT GAAAGAGGAA GCTAAAACCTC GAAACGAGGA CACAATTAAC
721 CAAGCAAAAAG CGAAAGTTAA GAGTGAACAA GCTGAGGCTA CAAGGTTAAA AAAAATCAAG
781 ACAGATCGTG AACAAGCTGA GGCTACAAGG TTAGAAAACA TCAAGACAGA TCGTGAAAAA
841 GCAGAAGAAG CTAACGAAA AGCAGAAGCA GAAGAAGTTA AAGATAAACT AAAGAGGCGG
901 ACAAACGAG CAGTTCCTGG AGAGCCAGCA ACACCTGATA AAAAAGAAAA TGATGCGAAG
961 TCTTCAGATT CTAGCGTAGG TGAAGAACT CTTCCAAGCC CATCCCTGAA ATCAGGAAAA
1021 AAGGTAGCAG AAGCTCAGAA GAAGGTAGCA GAAGCTGAGA AAAAAGCCAA
GGATCAAAAA
1081 GAAGAAGATC GCCGTAACCTA CCCAACCAAT ACTTACAAAA CGCTTGACCT TGAAATTGCT
1141 GAGTCCGATG TGAAAGTTAA AGAAGCGGAG CTTGAACCTAG TAAAAGAGGA
AGCTAAGGAA
1201 TCTCGAAACG AGGAAAAAGT TAAGCAAGCA AAAGCGAAAG TTGAGAGTAA AAAAGCTGAG
1261 GCTACAAGGT TAGAAAAAAT CAAGACAGAT CGTAAAAAAG CAGAAGAAGC TAAACGAAGA
1321 GCAGCAGAAG AAGATAAAGT TAAAGAAAAA CCAGCTGAAC AACCACAACC
AGCGCCGGCT
1381 CCTCAACCAG AAAAACCAAC TGAAGAGCCT GAGAATCCAG CTCCAGCTCC
AAACCTGAG
1441 AATCCAGCTG AACAACCAAA AGCAGAAAAA CCAGCTGATC AACAAGCTGA AGAAGACTAT
1501 GCTCGTAGAT CAGAAGAAGA ATATAATCGC TTGACTCAAC AGCAACCGCC AAAAAGTAA
1561 AAACCAGCAC AACCATCTAC TCCAAAAACA

//

FROM : FROMME LAWRENCE HAUG

PHONE NO. : 212 588 0500

Apr. 22 1999 06:02PM P3

PATENT

454312-3140

APR 23 P 12:21

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

(Includes reference to PCT International Applications)

& HAUG, LLP

FROMMER LAWRENCE & HAUG LLP

File No.: 454312-3140

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if plural, names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention ENTITLED:

**PNEUMOCOCCAL SURFACE PROTEIN C (PspC), EPITOPIC
REGIONS AND STRAIN SELECTION THEREOF, AND USES THEREFOR**

the specification of which:

☒ is attached hereto

was filed on _____ as:

United States Application Serial No: _____

☒ is based upon and claims priority from U.S. Provisional application Serial No. 60/082,728, filed April 23, 1998.

☒ with amendments through DATE EVEN HEREWITH (if applicable, give details).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119 (a) - (d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365 (a) of any PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT International applications designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign/PCT Application(s) [list additional applications on separate page]:

Priority Claimed:

Country (or PCT)Application Number:Filed (Day/Month/Year)YesNo

FROM : FROMMER LAWRENCE HAUG

PHONE NO. : 212 588 0500

Apr. 22 1999 06:03PM P4

PATENT
454312-3140

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

60/082,728 Filed April 23, 1998

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or § 365 (c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Prior U.S. (or U.S.-designating PCT) Application(s) [list additional applications on separate page]:
U.S. Serial No. Filed (Day/Month/Year) PCT Application No. Status (patented, pending, abandoned)

I hereby appoint Thomas J. Kowalski, Registration No. 32,147, and FROMMER LAWRENCE & HAUG, LLP or their duly appointed associates, my attorneys or agents, with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the Patent, and to transact all business in the Patent and Trademark Office and in the Courts in connection therewith, and to insert the Serial Number of the application in the space provided above, and specify that all communications about the application are to be directed to the following correspondence address.

Thomas J. Kowalski, Esq.
c/o FROMMER LAWRENCE &
HAUG, LLP
745 Fifth Avenue
New York, NY 10151
FAX (212) 588-0500

Direct all telephone calls to: (212) 588-0800
to the attention of:
Thomas J. Kowalski

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FROM : FROMME LAWRENCE HAUG

PHONE NO. : 212 588 0500

Apr. 22 1999 06:03PM P5

PATENT
454312-3140

INVENTOR(S):

Signature: Date: 4/23/99

Full name of sole or first inventor: BRILES, David E.

Residence: 760 Linwood Road, Birmingham, AL 35222

Citizenship: USA

Signature: Susan K. HollingsheadDate: 4/23/99

Full name of sole or second inventor: HOLLINGSHEAD, Susan K.

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Citizenship: USA

Full name of sole or third inventor: BROOKS-WALTER, Alexis

Residence: 2005 Russett Woods Trail, Birmingham, AL 35244

Citizenship: USA

Signature: Alexis Brooks-WalterDate: 4/23/99

Post Office Address(es) of inventors [if different from residence]:

NOTE: In order to qualify for reduced fees available to Small Entities, each inventor and any other individual or entity having rights to the invention must also sign an appropriate separate "Verified Statement (Declaration) Claiming [or Supporting a Claim by Another for] Small Entity Status" form [e.g. for Independent Inventor, Small Business Concern, Nonprofit Organization, Individual Non-Inventor].